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METTALOENZYMES AND THEIR MECHANISM IN HUMAN BODY

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INTRODUCTION

Transition metal chemistry is an integral part of coordination chemistry and its ever growing importance and applications encompass many fields. Especially since transition metals form an important part of bioinorganic chemistry, being essential to life, and also with the spawning of newer uses in organic synthesis. In our present study, we stress upon the following metals nickel, zinc, cadmium, cobalt, copper, manganese and vanadium. Nickel occurs in the oxidation states -1 to +4 with +2 being most common and relevant biologically. The overwhelming majority of Ni(II) complexes have coordination numbers four, five and six with three, seven and eight being rare. The geometries exhibited by nickel are square planar, tetrahedral, octahedral, trigonal bipyramidal and square pyramidal. Nickel plays a fundamental role in DNA and RNA metabolism. Zinc exhibits a rich variety of stereochemistry with geometries ranging from tetrahedral, square pyramidal, trigonal bipyramidal and octahedral. Zn(II) ion bound to imidazole ligands of aminoacid residue histidine form the biologically active zinc metalloprotein. Zn+2 complexes of ATP accelerate hydrolysis of polyphosphates and are of relevance to RNA and DNA polymerases. Zinc metalloenzymes participate directly in the catalytic process and serve stabilize the protein structure.

Cobalt forms a wide range of complexes with geometries ranging from octahedral, tetrahedral and square planar. It has oxidation states ranging from -1 to +5 with +2 and +3 being the most common. Complexes containing Co(II) are used as probes for active enzyme sites. Cobalt is present in the human body as Vitamin B12 (cyanocobalamin), a metallo complex.A review on biological importance of cobalt has been put forth by Nicholls.

Copper, a typical transition element, is one of the most abundant elements in the earth and occurs in a wide range of oxidation states ranging from 0 to + 4. The Cu(II) complexes exhibit coordination numbers four, five and six and are characterized by distortion (Jahn-teller distortion). Copper is an essential component of metalloenzymes and plays a vital role in structure and functions of nervous system and maintenance of skeletal and vascular system.

Manganese has a wide range of oxidation states from +7 to -3. Manganese acts as a redox catalyst in the photosynthetic process in plants. In living beings, its deficiency can lead to bone malformation, infertility and ataxia. Manganese catalyses many enzymes. Pyruvate carboxylase, a Mn+2 containing enzyme catalyses formation of oxaloacetate.

In our present study, we have synthesized the transition metal complexes of a few selected hydrazones. These. serve to illustrate the coordinating flexibility of the transition metals and also the control of stereochemistry of the complex by the ligands.

Metalloenzyme

Metalloenzymes are a subclass of metalloproteins that perform specific catalytic functions. Metallo enzymes Enzymes are molecules that catalyse reactions occurring in living cells. Specificity and efficiency are the important characteristics of an enzyme. Enzymes which are of interest to inorganic chemistry which are those which are composed of a protein structure called apoenzyme and a small prosthetic group ehich may be simple or complexed metal ion. A group that combines reversibly with an enzyme for a particular reaction and and is then released to combine with another enzyme is termed as coenzyme. The prosthetic group and the coenzyme are also called co-factor.

Transition state analogs play a key role in the competitive inhibition of metalloenzymes because they mimic the structure of the substrates transition state in the reaction of enzyme and substrate.

Metalloenzymes such as the ones containing zinc can also be regulated by diet. The source of zinc in humans is almost entirely through diet. Without proper intake of

metals such as zinc in a persons diet, the activity of the enzyme would be inhibited.

One thing to keep in mind while studying metalloenzymes is that they are incredibly diverse and function in a multitude of important physiological processes.

Enzymes name are derived by adding the suffix "ase" to the name of the process catalysed or the name of the molecules on which the enzyme act. The molecule on owhich the enzyme acts is called substrate.

Some important metallo-enzyme are listed below

- Alcohol dehydrogenase, alkaline phosphatase ,carbonic anhydrase and carboxy peptidase, all containing zinc.
- Arginase and oxaloacetate decarboxylase all containing mechanism
- Nitrogenase containing both iron and molybdenum.
- Phosphohydrolase and phosphotransferase, all containing magnesium.
- Cytochrome, peroxidise, catalases and ferridoxin, containing iron.
- Tyrosinase, amide oxidase, cytochrome oxidase, ascorbate oxidase and galactose oxidase all containing copper.

Structure and Overview

Metalloenzymes are proteins which function as an enzyme and contain metals that are tightly bound and always isolated with the protein. In proteins such as hemoglobins and cytochromes, the metal is Fe2+ or Fe3+, and it is part of the heme prosthetic group. In other metalloenzymes the metal is built into the structure of the enzyme molecule. The metal ion can not be removed with out destroying the structure of the enzyme. Metals built into the molecule include: most phosphotransferases, containing Mg2+; alcohol dehydrogenase, Zn2+; arginase, Mn2+; ferredoxin, Fe2+; and cytochrome oxidase, Cu2+.

Metals are usually found in the active site of the enzyme. The metals resemble protons (H+) in that they are electrophiles that are able to accept an electron pair to form a chemical bond. In this aspect, metals may act as general acids to react with anionic and neutral ligands.

Metal's larger size relative to protons is compensated for by their ability to react with more than one ligand. Metals typically react with two, four, or six ligands. A ligand is whatever molecule the metal interacts with. If a metal is bound with two ligands it will form a linear complex. If the metal reacts with four ligands the metal will be set in the center of a square that is planer or it will form a tetrahedral structure, and when six ligands react, the metal sits in the center of an octahedron.

By clicking the following image one can view a planar arrangement of and iron-porphyrin system: Amino acids in their peptide linkage in proteins possess groups with the ability to bind to the metal resulting in coordinate-covalent bonds. The free amino and carboxyl groups in a protein can bind to the metal and this may bind the protein to a specific, active conformation. The fact that metals bind to several ligands is important in that metals play a role in bringing remote parts of the amino acid sequence together and help establish an active conformation of the enzyme.

Zinc is the metal incorporated in carboxypeptidase A. The zinc atom serves as a metal ion catalyst and promotes hydrolysis. The substrate fits into the hydrophobic pocket in carboxypeptidase A and zinc binds to the carboxyl group of the substrate to help stabilize the enzyme-substrate complex. In this example the zinc ion acts a generalized acid and stabilizes the developing O- as water attacks the carbonyl.

Zinc can also perform a different role in enzymes like the role it performs in carbonic anhydrase. Here the metal binds H2O and makes it acidic enough to lose a proton and form a Zn-OH group. The zinc metal serves as a nucleophile to the substrate. Since zinc has the ability to act as an electrophile or as the source of a nucleophilic group it is incorporated and used by many enzymes.

Bulk Metals & Trace Metals

There are many elements which are essential for biological reactions. Some of them are required in relatively large quantities and therefore called **macronutrients or bulk elements**. These elements are **Na, K, Mg, Ca, P, S, Cl** along with four most abundant element C,H,N,O in biological system. There are also some elements which are required in small amounts , called **trace elements** or **micronutrients**. these elements are : all the first row transistion metals (except Scand Ti) Mo and W (from second and third row transistion metal respectively)and non –metals (B,Si,Se,F,and I). Ni ,Cd, Pd and Asare ultra trace element and are essential at very low concentration.

The elements are toxic at concentration above ultra trace level. Essential trace elements play an important role as a cofactor for certain enzymes involved in metabolism and cell growth, most of them involved in the metabolism of proteins, carbohydrates, lipids, and energy. They are also necessary for growth, development, muscle and nerve function, normal cellular functioning, and synthesis of some hormones and connective tissue.

The role of trace elements in biological processing may provide vital clue for understanding the etiology of some diseases such as cancer. The ability of trace elements to function as substantial affecter in a variety of the processes necessary for life, such as regulating homeostasis and prevention of free radical damage, can provide an answer to the definite correlation between content of trace elements and many common diseases. In the past ten years, studies have focused extensively on

determine the levels of trace elements in cancers patients, as an attempt to understand the nature of relationships between cancer and trace elements. Thus, the expected role of trace elements will enable to understand the etiopathogenesis of cancer and provide a rapid diagnostic facility and also create effective treatment modalities. Essential inorganic elements and their role in biology.

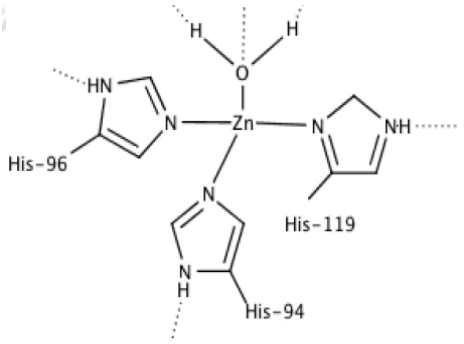
Metal	Function
Sodium	Charge carrier; osmotic balance
Potassium	Charge carrier; osmotic balance
Magnesium	Structure; hydrolase; isomerise
Calcium	Structure: trigger; charge carrier
Vanadium	Nitrogen fixation; oxidase
molybednum	Nitrogen fixation; oxidase; oxo transfer
Manganese	Photosynthesis; oxidase; structure Iron Oxidase; dioxygen tramped and storage; electron transfer: nitrogen fixation
Cobalt	Oxidase; alkyl group transfer
Nickel	Hydrogenase; hydrolase
Copper	Oxidase; dioxygen transport: electron transfer
Zinc	Structure; hydrolase
Tungsten	Dehydrogenase

Carbonic anhydrase: The first zinc metalloenzyme, carbonic anhydrase was discovered in 1940 by Keilin and Mann. It is a lyase that catalyzes the reversible hydration of carbon dioxide to form the bicarbonate ion
$CO2 + H2O \rightleftharpoons HCO3^- + H^+$

In the absence of the enzyme this reaction occurs relatively slowly at physiological pH and the presence of CA increases the rate upto a million fold. In vitro it catalyzes several other reactions also such as the hydrolysis of esters and the hydration of aldehydes.

Carbonic anhydrases are found in all kingdoms of life, occurring in animals, plants and several bacteria. They play an essential role in biological processes like respiration, photosynthesis and in maintaining pH. There are several forms of CA. All the forms are single chain polypeptides with molar mass about 30,000 Da and have one zinc ion per molecule. High-activity forms have kcat~106 s-1 at 25o C and are labeled II. Low- activity forms and the very-low-activity forms have kcat~105 s-1 and 103 s-1 and are labeled as I and III respectively. The best studied carbonic anhydrase is CA II from red blood cells.

Active site of human carbonic anhydrase II (HCA II)
The active site of human carbonic anhydrase II is located at the bottom of a cleft in the enzyme. The protein is composed of 259 amino acids. HCA II contains an important cofactor at the active site, namely, dipositive zinc ion, that helps activate water molecules prior to their reaction with CO2. This zinc is coordinated to the imidazole nitrogen atoms of three histidine residues. Histidine-94, histidine-96 are ligated to zinc via their Nε atoms and histidine-119 is bound via Nδ atom. The three histidine NH protons are all engaged in hydrogen bonding. Histidine-94, histidine-96, histidine-119 are involved in hydrogen bonding with glutamine-92, asparagine-244 and glutamate-117 respectively. The fourth coordination site of zinc is occupied by a water molecule (Figure 5) to give a distorted tetrahedral geometry. The active site pocket also contains ordered network of water molecules and other amino acids like threonine-199, glutamate-106, histidine-64 that are important for hydrogen bonding, proton transfer (rate determining step in catalytic mechanism) and for binding the CO2 substrate.



ure 5: Active site of Human Carbonic Anhydrase (II)

Zinc(II) is a Lewis acid. Zinc ion present in HCA(II) is more acidic than the zinc in CPDA. The difference is because of the ligands coordinated to zinc. CPDA has two histidines and a bidentate glutamate ion ligated to zinc. HCA(II) has three histidines. Histidine being neutral and less basic than glutamate anion, makes the zinc more acidic in HCA(II). The binding of water to zinc, reduces the pKa of water from its normal 14 down to 7. This leads to its deprotonation at neutral pH to form the strong hydroxide nucleophile (Figure 6).

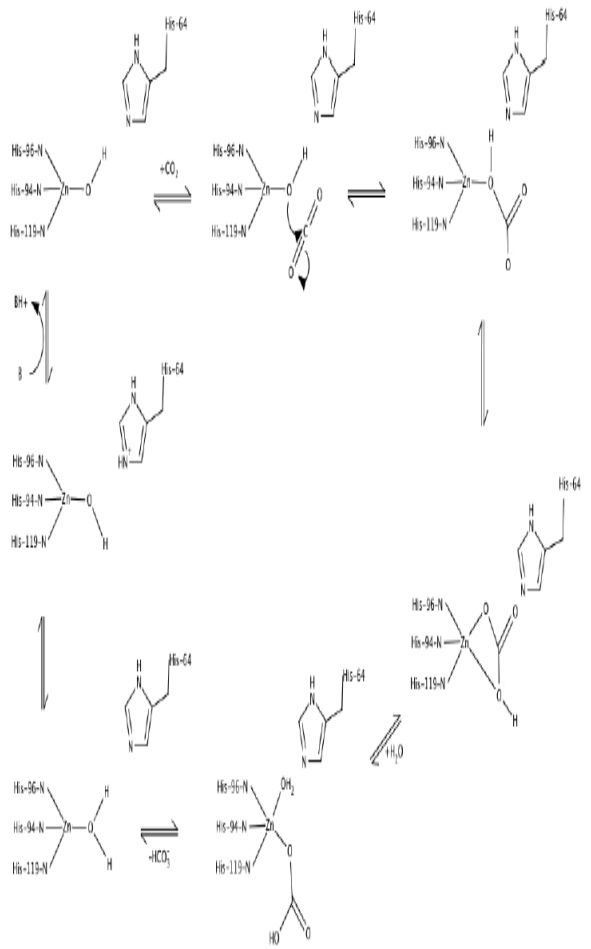


Figure 6: Catalytic mechanism of human carbonic anhydrase (II)

The dissociated proton is transferred indirectly to histidine-64 via water molecules. Histidine-64 shuttles protons from the active site to the buffer in solution, shifting the equilibrium in the forward direction in favor of the formation of hydroxide ion. The enzyme then positions carbon dioxide for nucleophilic attack by the hydroxide. The nucleophilic attack by hydroxide on carbon dioxide produces a zinc bound bicarbonate. Addition of water displaces the bicarbonate ion, completing the cycle.

Carboxypeptidase A (CPDA)

Carboxypeptidase A is one of the most studied peptide hydrolyzing enzyme. In 1929 Waldschmidt – Leitz and Purr isolated CPDA for the first time. It was the first metalloprotease and second zinc enzyme to be identified.

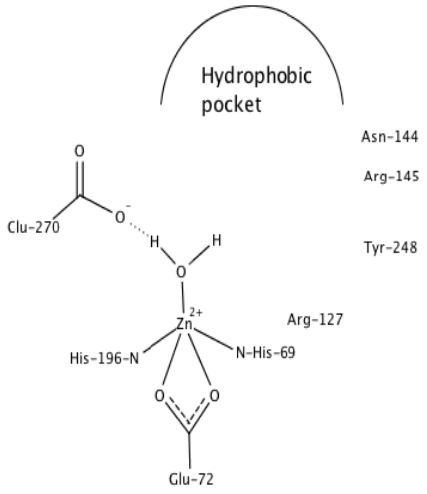
There are two kinds of carboxypeptidases. Enzymes of one class are not metalloenzymes. Yeast carboxypeptidase C belongs to this class. The second class of carboxypeptidases are metalloenzymes. Carboxypeptidase A (CPDA) is an example of this class of enzyme. It is an exopeptidase (i.e. one which cleaves only terminal peptide bonds) that hydrolyses a peptide bond at carboxyl terminal (C- terminal) end of a peptide or protein. A related protein carboxypeptidase B catalyzes the hydrolysis of basic amino acids lysine, arginine, ornithine from C-terminal position of polypeptides. Hydrolysis by CPDA occurs only if the C-terminal residue has S- configuration and its carboxyl group is free. Peptides possessing any C- terminal residue except proline will be hydrolysed and an aromatic amino acid side chain at the C- terminal residue is favoured in the substrate.

There are three forms of bovine pancreatic carboxypeptidase A; Aα, Aβ and Aγ ; with 307, 305 and 300 amino acid residues respectively. The Aβ and Aγ are shortened at the N- terminus. The reaction for hydrolysis of peptides by CPDA is R-CO-NH-CHR'-COO- + H2O ⇌ R-COO- + H3N+-CHR'-COO-

At neutral pH, in the absence of enzyme, the above reaction is slow with rate constants of the order of 10 -11 s -1. However, if the above reaction is catalyzed by carboxypeptidase the rate constants can attain values of 10 4 s -1. CPDA is initially produced in an inactive form (zymogen), procarboxypeptidase A, in the pancreatic juice of animals. The inactive form is converted to the active CPDA by the enzyme enteropeptidase.

Active site of Carboxypeptidase A

CPDA consists of a single protein chain (M= 34,300) and has one Zn 2+ ion present in the active site. It is ellipsoidal with a cleft on one side that contains the zinc ion. In the native enzyme, the zinc coordination number is five (imidazole nitrogens from two histidine residues, histidine- 69 and histidine-196, two carboxylate oxygens of glutamate–72 and a water molecule) with a distorted pentacoordination geometry (Figure 2). Many acidic and basic amino acids namely asparagine-144, arginine-145, tyrosine-248, arginine-127 and glutamate-270 are also present in the active site and are important for the activity of the enzyme. The active site cavity has a hydrophobic pocket that accommodates the non-polar R' of the C- terminal amino acid of the substrate undergoing hydrolysis.



Active site of carboxypeptidase A

Catalytic Mechanism of Carboxypeptidase A

The mechanism for hydrolysis of peptides or proteins by carboxypeptidase A was proposed by Christianson and Lipscomb. In this mechanism, the water molecule coordinated to zinc is important for the catalytic action. The bound water is converted to nucleophilic hydroxide, by interaction with glutamate-270 (Figure 3), which then attacks the carbonyl carbon of the scissile peptide bond.

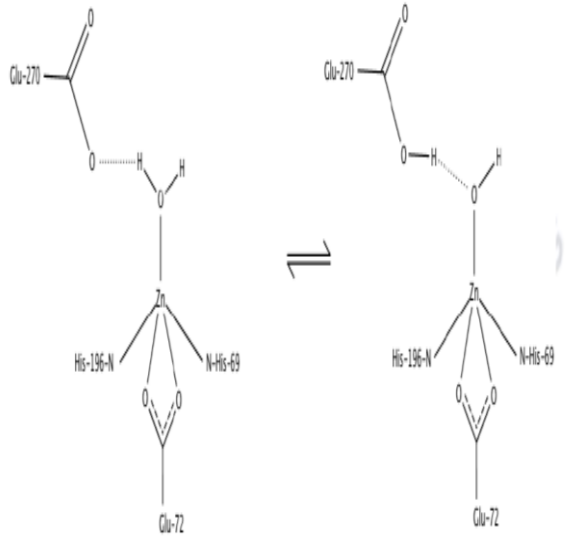


Figure. 3: Interaction of coordinated water with glutamate-270 present in the active site of CPDA.

The first step is positioning of the substrate on the enzyme Figure 4(b). The enzyme interacts with the substrate via hydrogen bonds (arginine-145 and asparagine-144 with terminal carboxylate group, tyrosine-248 with the penultimate peptide NH, arginine-127 with carbonyl oxygen of the terminal peptide bond) and van der Waals attraction if the terminal group is aromatic (hydrophobic pocket with hydrophobic R' of C-terminal amino acid). The interaction of arginine-127 with the carbonyl of the terminal peptide bond increases the Lewis acidity of the latter making it more susceptible for nucleophilic addition. Then the water molecule bound to zinc performs a nucleophilic attack on the the carbonyl carbon of the C- terminal peptide bond with the simultaneous transfer of a proton to glutamate-270. At this point the glutamate-72 bound to zinc ion becomes monodentate from bidentate, maintaining the five coordination of zinc ion. This process is called carboxylate shift. The tetrahedral intermediate formed by the nucleophilic attack is shown in Figure 4(c). The protonated glutamate-270 now transfers its proton to the amide group of the terminal peptide bond. This causes the collapse of the tetrahedral intermediate resulting in the formation of the free carboxyl terminal amino acid and the shortened peptide chain with its terminal carboxylate group bound to zinc Figure 4(d). A water molecule adds to the zinc ion. Finally the cleaved peptide leaves and zinc ion gets back to its original pentacoordination.

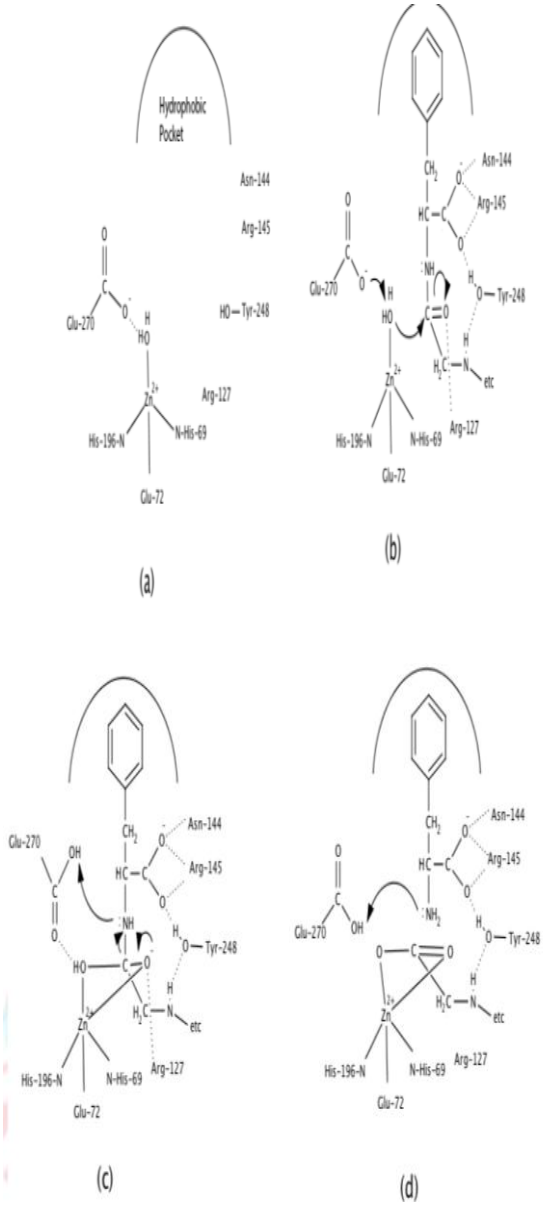


Figure. 4: Mechanism of hydrolysis of a peptide by Carboxypeptidase A.

Superoxide dismutase

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. One of the exceedingly rare exceptions is Lactobacillus plantarum and related lactobacilli, which use a different mechanism.

Reaction

The SOD-catalysed dismutation of superoxide may be written with the following half-reactions

• $M(n+1)+\text{-SOD} + O2 - \rightarrow Mn+\text{-SOD} + O2.$

• $Mn+\text{-SOD} + O2 - + 2H+ \rightarrow M(n+1)+\text{-SOD} + H2O2.$

where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

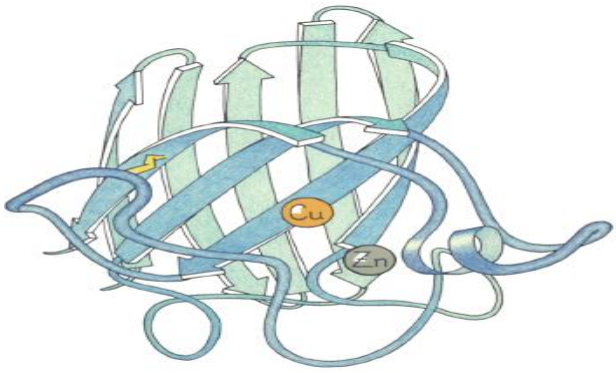
In this reaction the oxidation state of the metal cation oscillates between n and n+1.

Types

General: Irwin Fridovich and Joe McCord discovered the activity of superoxide dismutase. SOD's were previously known as a group of metalloproteins with unknown function; for example, CuZnSOD was known as erythrocuprein and as the veterinary antiinflamatory drug "Orgotein". Likewise, Brewer (1967) identified a protein that later became known as superoxide dismutase as an indophenol oxidase by protein analysis of starch gels using the phenazine-tetrazolium technique.

Several common forms of SOD exist: they are proteins cofactored with copper and zinc, or manganese, iron, or nickel. Thus, there are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and the Ni type, which binds nickel.

• **Copper and zinc** – most commonly used by eukaryotes. The cytosols of virtually all eukaryotic cells contain an SOD enzyme with copper and zinc (Cu-Zn-SOD). For example, Cu-Zn-SOD available commercially is normally purified from the bovine erythrocytes: The Cu-Zn enzyme is a homodimer of molecular weight 32,500. **The bovine Cu-Zn protein** was the first SOD structure to be solved, in 1975. It is an 8-stranded "Greek key" beta-barrel, with the active site held between the barrel and two surface loops. The two subunits are tightly joined back-to-back, primarily by hydrophobic and some electrostatic interactions. The ligands of the copper and zinc are six histidine and one aspartate side-chains; one histidine is shared between the two metals.



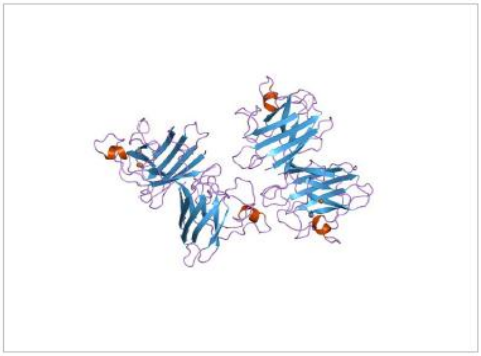
Bovine Cu-Zn SOD subunit.[21]

• Iron or manganese – used by prokaryotes and protists, and in mitochondria.

• Iron – E. coli and many other bacteria also contain a form of the enzyme with iron (Fe-SOD); some bacteria contain Fe-SOD, others Mn-SOD, and some contain both. (For the E. coli Fe-SOD). Fe-SOD can be found in the plastids of plants. The 3D structures of the homologous Mn and Fe superoxide dismutases have the same arrangement of alpha-helices, and their active sites contain the same type and arrangement of amino acid side-chains.

• Manganese – Chicken liver (and nearly all other) mitochondria, and many bacteria (such as E. coli), contain a form with manganese (Mn-SOD): for example, the Mn-SOD found in human mitochondria. The ligands of the manganese ions are 3 histidine side-chains, an aspartate side-chain and a water molecule or hydroxy ligand, depending on the Mn oxidation state.

Copper/zinc superoxide dismutase



Structure of the yeast Cu,Zn enzyme superoxide dismutase.[27]

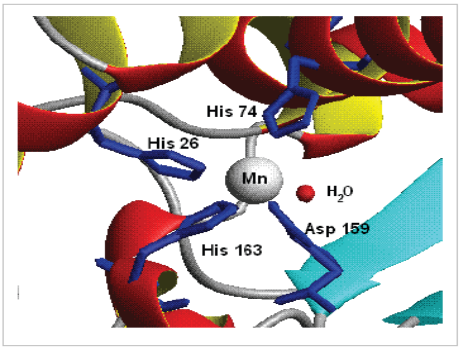
Human

Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. The genes are located on chromosomes 21, 6, and 4, respectively.

SOD1, soluble

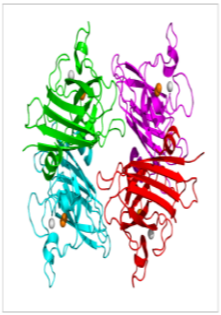
Crystallographic structure of the human SOD1 enzyme (rainbow colored N-terminus = blue, C-terminus = red) complexed with copper (blue-green sphere) and zinc (grey spheres).[59]

SOD2, mitochondrial



Structure of the active site of human superoxide dismutase 2.[1]

SOD3, extracellular



Crystallographic structure of the tetrameric human SOD3 enzyme (cartoon diagram) complexed with copper and zinc cations (orange and grey spheres respectively).[73]

Human white blood cells generate superoxide and other reactive oxygen species to kill bacteria. During infection, some bacteria (e.g., Burkholderia pseudomallei) therefore produce superoxide dismutase to protect themselves from being killed.

Biochemistry

Simply stated, SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin forbidden. In biological systems, this means its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O2−) spontaneously dismutes to O2 and hydrogen peroxide(H2O2) quite rapidly (~105 M−1s−1 at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts the NO radical, and makes toxic peroxynitrite. The dismutation rate is second order with respect to initial superoxide concentration. Thus, the half-life of superoxide, although very short at high concentrations (e.g., 0.05 seconds at 0.1mM) is actually quite long at low concentrations (e.g., 14 hours at 0.1 nM). In contrast, the reaction of superoxide with SOD is first order with respect to superoxide concentration. Moreover, superoxide dismutase has the largest kcat/KM (an approximation of catalytic efficiency) of any known enzyme (~7 x109 M−1s−1), this reaction being only limited by the frequency of collision between itself and superoxide. That is the reaction rate is "diffusion limited". Even at the subnanomolar concentrations achieved by the high concentrations of SOD within cells, superoxide inactivates the citric acid cycle enzyme aconitase, can poison energy metabolism, and releases potentially toxic iron. Aconitase is one of several iron-sulfur containing (de)hydratases in metabolic pathways shown to be inactivated by superoxide.

Physiology

Superoxide is one of the main reactive oxygen species in the cell. Consequently, SOD serves a key antioxidant role. The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amid massive oxidative stress Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma,[87] an acceleration of.[86] age-related muscle mass loss,[88] an earlier incidence of cataracts and a reduced lifespan. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan, though they are more sensitive to hyperoxic injury.[89 Knockout mice of any SOD enzyme are more sensitive to the lethal effects of superoxide generating drugs, such as paraquat and diquat Drosophila lacking SOD1 have a dramatically shortened lifespan whereas flies lacking SOD2 die before birth. SOD knockdowns in C. elegans do not cause major physiological disruptions. Knockout or null mutations in SOD1 are highly detrimental to aerobic growth in the yeast Sacchormyces cerevisiae and result in a dramatic reduction in post-diauxic lifespan. SOD2 knockout or null mutations cause growth inhibition on respiratory carbon sources in addition to decreased post-diauxic lifespan. Several prokaryotic SOD null mutants have been generated, including E. Coli. The loss of periplasmic CuZnSOD causes loss of virulence and might be an attractive target for new antibiotics.

Role in disease

Mutations in the first SOD enzyme (SOD1) can cause familial amyotrophic lateral sclerosis (ALS, a form of motor neuron disease). The most common mutation in the U.S. is A4V, while the most intensely studied is G93A. The other two isoforms of SOD have not been linked to any human diseases, however, in mice inactivation of SOD2 causes perinatal lethalityand inactivation of SOD1 causes hepatocellular carcinoma.Mutations in SOD1 can cause familial ALS (several pieces of evidence also show that wild-type SOD1, under conditions of cellular stress, is implicated in a significant fraction of sporadic ALS cases, which represent 90% of ALS patients.),[94] by a mechanism that is presently not understood, but not due to loss of enzymatic activity or a decrease in the conformational stability of the SOD1 protein. Overexpression of SOD1 has been linked to the neural disorders seen in Down

syndrome. In recent years it has become more apparent that in mice the extracellular superoxide dismutase (SOD3, ecSOD) is critical in the development of hypertension.[96][97] In other studies, diminished SOD3 activity was linked to lung diseases such as Acute Respiratory Distress Syndrome (ARDS) or Chronic obstructive pulmonary disease (COPD).

Superoxide dismutase is also not expressed in neural crest cells in the developing fetus. Hence, high levels of free radicals can cause damage to them and induce dysraphic anomalies (neural tube defects).

Pharmacological activity

SOD has powerful antinflammatory activity. For example, SOD is highly effective in treatment of colonic inflammation in experimental colitis. Treatment with SOD decreases reactive oxygen species generation and oxidative stress and, thus, inhibits endothelial activation and indicate that modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease. Similarly, SOD has multiple pharmacological activities. E.g., it ameliorates cis-platinum-induced nephrotoxicity in rodents.[102] As "Orgotein" or "ontosein", a pharmacologically-active purified bovine liver SOD, it is also effective in the treatment of urinary tract inflammatory disease in man.[103] For a time, bovine liver SOD even had regulatory approval in several European countries for such use. This was truncated, apparently by concerns about prion disease. An SOD-mimetic agent, TEMPOL, is currently in clinical trials for radioprotection and to prevent radiation-induced hair-loss. TEMPOL and similar SOD-mimetic nitroxides exhibit a multiplicity of actions in diseases involving oxidative stress. For a review, see Wilcox.

Cosmetic uses

SOD may reduce free radical damage to skin—for example, to reduce fibrosis following radiation for breast cancer. Studies of this kind must be regarded as tentative, however, as there were not adequate controls in the study including a lack of randomization, double-blinding, or placebo. Superoxide dismutase is known to reverse fibrosis, perhaps through reversion of myofibroblasts back to fibroblasts.

Urease

Urease belongs to the super family of amidohydrolases and phosphotriestreases. Urease involves the nitrogen metabolism; urea degradation; generating CO_2 and NH_3 from urea. Urease has wide clinical applications. Urease enzyme serves as a virulence factor and is responsible for **pathogenesis in humans** It is essential in colonization of a host organism and in maintenance of bacterial cells in tissues. Urease activity of microbial sources has contributed to the development of many diseases and urease from plant sources is used as vaccine against microbial infection on the basis of its inhibitory activity. Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates, as well as in soils, as a soil enzyme. They are nickel containing metalloenzymes of high molecular weight.

These enzymes catalyze the hydrolysis of urea into carbon dioxide and ammonia

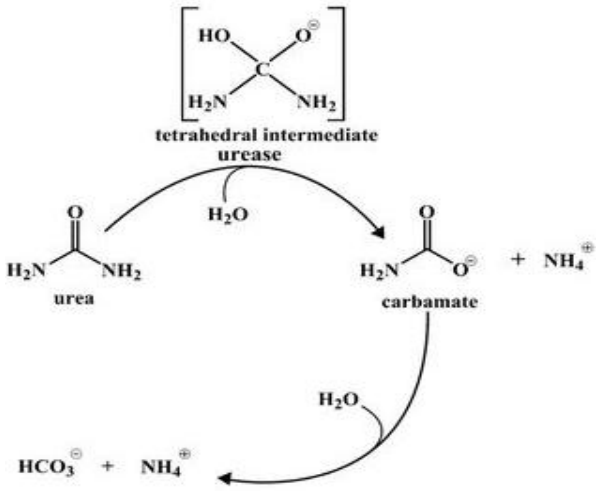
$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$

The hydrolysis of urea occurs in two stages. In the first stage, ammonia and carbamate are produced. The carbamate spontaneously and rapidly hydrolyzes to ammonia and carbonic acid. Urease activity increase the pH of its environment as it produces ammonia, which is basic. Urease is also found in mammals and humans which is considered to be very harmful to mammals due to production of the toxic ammonia product in the mammalian cells. However, mammalian cells does not produce urease, in fact, the source are the ammonia product in the mammalian cells. However, mammalian cells does not produce urease, in fact, the source are the ammonia product in the mammalian cells. However, mammalian cells does not produce urease, in fact, the source are the to have high urease activity in its large intestine, a part of gastrointestinal tract.Previously, other mammals i.e. rats, pigs and rabbits, with postgastric fermentation were detected with lower urease activity compared to European Hare **In human kidneys**, urea is present in order for everyday functions and is estimated that per day, a healthy adult excretes about 10 to 30 g of urea. Other than urea being found in urine, it is also present in sweat, blood serum and stomach. Inside the mitochondria of a liver cell, excess ammonia is converted to urea through the urea cycle and if some excess ammonia is still present in the mitochondria, then it gets used up for protein synthesis. There are specific tissues involved during urea processing which are epithelial, extrahepatic and muscle tissues. With the production of ammonia and amino acids, the cell proteins are broken down by proteolytic enzymes already present in the muscle tissue. Similarly, identical cell proteins are predicted to convert previously broken down ammonia into urea.Once the urea is formed in the liver, it is excreted through urine after passing from bloodstream and the kidneys.

History

Its activity was first identified in 1876 by Frédéric Alphonse Musculus as a soluble ferment. In 1926, James B. Sumner, showed that urease is a protein by examining its crystallized form.Sumner's work was the first demonstration that a protein can function as an enzyme and led eventually to the recognition that most enzymes are in fact proteins. Urease was the first enzyme crystallized. For this work, Sumner was awarded the Nobel prize in chemistry in 1946.The crystal structure of urease was first solved by P. A. Karplus in 1995.

Urease is capable of urea hydrolysis. This compound is widespread: it is found in the natural environment (water and soil) and in human body, where its occurrence is connected with protein degradation. In humans, urea is a factor of normal functions of kidneys. A healthy adult excretes about 30 g of urea per day. However, it is present not only in urine, but also in blood serum, sweat and even in stomach. Hydrolysis of urea by urease is a complex process. In the first step, one molecule of ammonia and one molecule of carbamate appear. In water solution, carbamate spontaneously converts into the second ammonia molecule and carbonic acid. Next ammonia is protonated. This process results in pH increase Urease and ammonia, generated during urea hydrolysis, may be toxic for human tissue and probably have role in long-lasting diseases, like atherosclerosis or rheumatoid arthritis.



Scheme of urea hydrolysis

Genetic and structural organization of bacterial urease

Urease is a nickel-containing enzyme, which requires activity of a few additional proteins for acquisition of its hydrolytic properties. This process involves genes coding structural enzyme polypeptides as well as genes coding accessory proteins, located in a joint cluster.

Bacterial ureases are always multimeric enzymes composed of two or three different polypeptides. In P. mirabilis, three structural subunits: 11 kDa UreA (subunit γ), 12.2 kDa UreB (subunit β) and 61 kDa UreC (subunit α) are found. These polypeptides are encoded by three structural genes: ureA, ureB and ureC respectively. Such organization is characteristic of most pathogenic and environmental bacteria. Unique urease of Helicobacter sp. has a different structure. In H. pylori, urease consists of only two subunits: 26.5 kDa UreA (subunit β) and 61.7 kDa UreB (subunit α) coded by ureA and ureB genes. A smaller Helicobacter sp. urease structural gene (ureA) corresponds with a hypothetical fusion gene arisen from ureA and ureB typical of other bacteria, while a larger gene (ureB) is analogous to ureC (Fig. **2**).

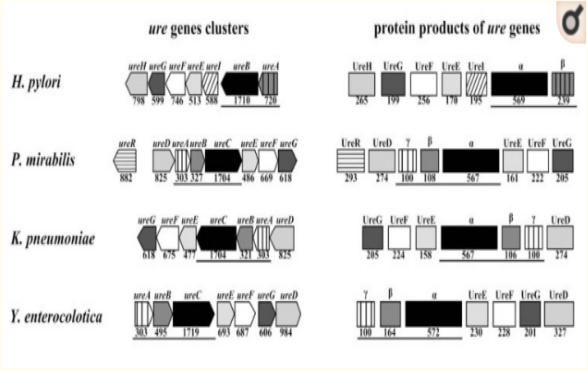


Fig. (2)

Scheme of genetic organization of urease genes and structural composition of urease.

Genetic organization of *ure* genes was performed basing on Microbial Genome Viever MGV 2.0 (http://mgv2.cmbi.ru.nl) for *H. pylori* G27, *P. mirabilis* HI4320, *K. pneumonia* 342 and *Yersinia enterocolitica* 8081. Under genes/polypeptides are sizes of particular genes as well as a number of amino acids of particular polypeptide were taken from NCBI database for records: CP001173 (*H. pylori* G27), AM942759 (*P. mirabilis* HI4320), NC_011283 (*K. pneumonia* 342) and NC_008800 (*Y. enterocolitica* 8081); structural genes as well as urease subunits are underlined.

Urease composed of two different polypeptides (21 kDa and 65 kDa) was also identified in SL100 ureolytic coccoid strain isolated from stomach biopsy material. This strain was related to *Staphylococcus cohnii* and *Staphylococcus xylosus*, which possess three urease subunits.

An active center of enzyme with two metal ions is located in the largest of structural subunits. In all ureases it is designed as UreC, except *Helicobacter* sp., in which case it is UreB. Ureases are nickel-containing enzymes; however, for microaerophilic *Helicobacter mustelae* an iron-containing urease was revealed.

All bacterial ureases occur as inactive apoenzymes composed of three or two types of polypeptides coded by specific structural genes. However, additional proteins, products of accessory genes are required for urease activation. Those proteins (UreD, UreE, UreF, UreG and UreH) are involved in transporting nickel ions into a cell and in incorporating them into an active center of apoenzyme. *P. mirabilis* produce active urease in presence of urea. In these bacteria a regulatory gene *ureR* is present (see Fig. **2**). Its product is a urea inducible regulator controlling expression of remaining genes.

A highly mobile helix-turn-helix motif, located in α subunit and called "flap" is essential for urease activity (see Fig.5). It may adopt two different conformations. In the "open" position, urea may enter into the active site,

where hydrolyze is performed. In the "closed" position, flap covers the active center and blocks access to it.

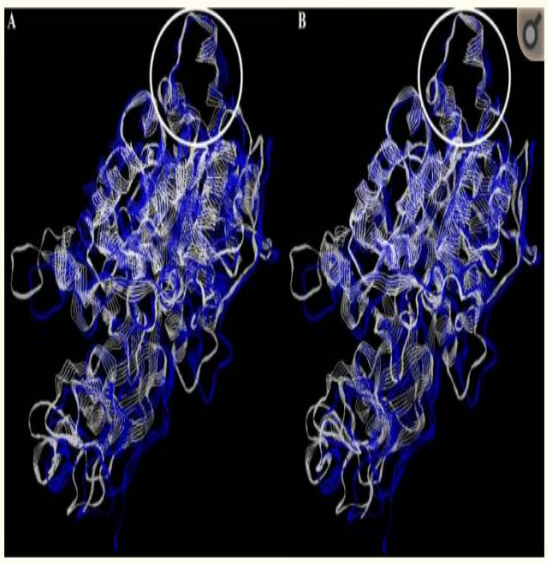


Fig. (5)

Conformational conservatism of bacterial ureases. All models of bacterial ureases were from ExPASy SIB Bioinformatics Resource Portal (Q7X3W5 - *H. pylori*; P16122 - *P. hauserii*; Q6GEE4 - *S. aureus*); overlapping was performed with RasWin Molecular Graphics Visualisation Tool (http://rasmol.org/). A - structure of *H. pylori* (blue) and *P. hauserii* (white) ureases, B - structure of *H. pylori* (blue) and *S. aureus* (white) ureases; flap region is marked by a white ellipse.

Active ureases are heterooligomeric complexes. However, the number of particular structural subunits is always equal. In *K. aerogenes* urease, as well as in other tree-subunit bacterial ureases, UreC/UreB/UreA molecules occur in the ratio 1:1:1. Likewise, for *Helicobacter* sp. UreB/UreA are always in the ratio 1:1.

Urease from *K. aerogenes*, as well as the most of other bacteria, is triple trimer $(\alpha\beta\gamma)_3$ with three active centers, one in each of three α subunits. Amino- and carboxyl terminus of each subunit are free and they are able to bind additional compounds without disturbing the enzyme structure. But *Prochrorococcus marinus* sp. PCC 9511 produces urease composed according to $(\alpha\beta\gamma)_2$ pattern. Enzymes from *Helicobacter* sp. may form a more complex structure, built from 12 subunits. Polypeptides α and β are linked forming trimer $(\alpha\beta)_3$, where N-terminal domain of β subunit are essential in aggregation process. Then, four such trimers form a tetrahedral complex (Fig **3**).

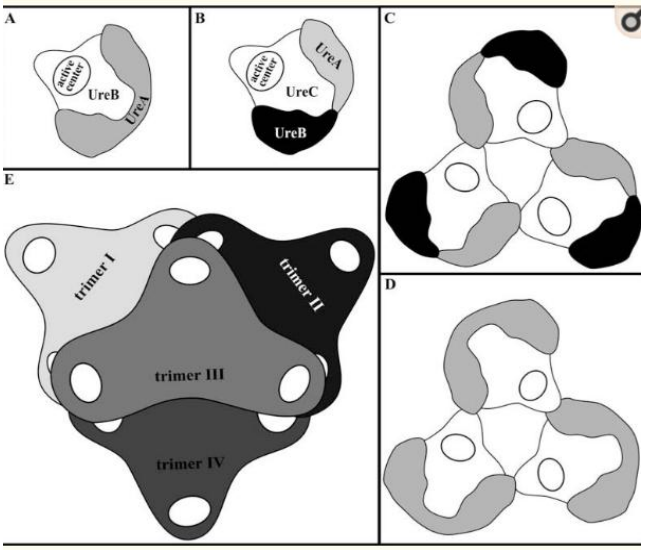


Fig. (3)

Scheme of structure of bacterial ureases. Domain organization is reported for (A) *H. pylori* and (B) *Bacillus pasteurii* urease monomers, (C) *B. pasteurii* and (D) *H. pylori* urease trimers, and (E) *H. pylori* dodecamer.

Urease as a pathogenic bacterial virulence factor

Bacterial ureases play a role in disease pathogenesis. They are connected with urinary stones occurrence and catheters blocking, pyelonephritis, ammonia encephalopathy, hepatic coma as well as gastritis. In many papers there are information concerning toxic effects of bacterial ureases (Table **3**).

Table 3.

Pathologic Effect of Bacterial Ureases in Human Diseases.

Role of urease	Bacterium species	Disease
Surviving in host's organism	*H. pylori*	gastritis, peptic ulcers
	M. tuberculosis	tuberculosis
	E. coli	hemorrhagic colitis, HUS
Persistence to host's cells	*H. pylori*	gastritis, peptic ulcers
Precipitation of polyvalent ions	*P. mirabilis, M. morganii, U. urealyticum* and others	urinary tract infections
Stimulation of inflammatory reaction	*H. pylori*	gastritis, peptic ulcers
	Y. enterocolitica	reactive arthritis
Cytotoxic effect on host's cells	*H. pylori*	gastritis, peptic ulcers
Damage to glycosaminoglycan layer	*P. mirabilis*	urinary tract infections
Damage of tight junctions	*H. pylori*	peptic ulcers
Aggregation of blood platelets	*H. pylori*	gastritis, cardiovascular disease

HUS - hemolytic uremic syndrome

The role of urease in bacterium surviving in unfavorable microenvironment in the host's body is especially noticeable in case of *H. pylori*, a causative agent of gastritis and peptic ulceration. At *in vitro*conditions, *H. pylori* is sensitive to low pH. During infection, microorganisms have to pass through gastric acid before reaching the protective mucus layer. In these circumstances, a pathogen produces a large amount of urease which is not observed in other bacteria. At low pH, enzymatic activity of *H. pylori*urease is probably connected with its dodecameric structure. This enzyme is also able to perform a more efficient hydrolysis of urea. This property may be due to mobility of the flap region, which is different than in *K. aerogenes* or *B. pasteurii* ureases. Due to the high activity of *H. pylori* urease, local microenvironment surrounding bacterium becomes nearly neutral. Moreover, live bacterial cells adsorb on the surface enzymes released upon other *H. pylori* autolysis, which makes it possible for them to get to gastric mucus layer safely. Ureolytic activity is essential for surviving *M. tuberculosis*, an etiologic factor of tuberculosis, a long-lasting inflammatory lung disease. Bacteria infect macrophages. They reside in phagosome, where alkalization due to ureolytic activity and subvert phagosome maturation takes place. Additionally, urease activity enables bacterium to exist in the environment where nitrogen sources are limited to urea. Ureolytic activity is useful in better surviving of bacteria also in case of uropathogenes. Urease facilitates urinary tract infection. Infection dose for ureolytic *P. mirabilis* HI4320 was 1000-times lower in comparison with its non-ureolytic mutant. Urease activity raises pH of human urine, which allows precipitation of normally soluble polyvalent ions to struvite and carbonate apatite. These compounds aggregate around bacteria, forming urinary stones. Inside such stones, microorganisms are protected from antibiotics and the host's immune system. Urinary stones block urethra or catheters leading to acute bacteriuria. The role of ureolytic activity in urinary stones formation was also showed for *U. urealyticum*, *S. saprophiticus*, *S. aureus* and some *Klebsiella* spp., *Pseudomonas* spp., as well as *Corynebacterium* sp. D2, *P. penneri*, *P. stuartii*, *M. morganii*.

One of the features essential in bacterial infections is persistence to the host's cells. Schoep *et al.* showed that *H. pylori* urease have two sites (one at the N-termini of UreA subunit and the other at C-termini of UreB) which were involved in persistence to endothelial cells during mouse colonization. This observation was confirmed by investigations with urease-negative *H. pylori* mutants incapable of colonization. Moreover, also urease released from lyzed bacterial cells is capable of adsorption into the mucus layer.

Bacterial ureases affect host immune system cells. In *H. pylori* infection, this metalloenzyme activates monocytes and neutrophils, which leads to secretion of inflammatory cytokines and causes indirect damage to epithelial cells. Urease is a chemotactic factor for monocytes and neutrophils. Inflammatory reaction may also be initiated by adsorption of released enzyme into the mucus layer. Induction of inflammatory reaction was also observed for *Y. enterocolitica* urease. Ability of bacterial UreB subunit to induce experimental reactive arthritis was revealed.

Urease may contribute in damaging host's cells. Enzyme from *H. pylori* stimulates expression of inducible NO-synthesizing enzyme (iNOS), which may have a cytotoxic effect. Urease may exert a toxic effect also indirectly, by ammonia - the product of urea hydrolysis. During *H. pylori* infection, a stimulation of an oxidative burst in neutrophils ensues and there is a release of hydrogen peroxide, which next oxidizes chlorine ions. Ammonia generated by urease reacts with them and gives toxic monochloramine. Johnson *et al.* revealed, using mouse model, that ammonia causes tissue damage also during urinary tract infections with ureolytic *P. mirabilis*. In kidneys, an acute inflammation as well as necrotic cells were observed. After one week, pyelonephritis was in progress. Struvite stones were noted. After two weeks, kidneys were ulcerated and fibrosis was visible. Moreover, ammonia released by urease causes damage to the glycosaminoglycan layer in urothelial surface, and disturbs its protective function.

Recently, a new role of *H. pylori* urease has been established. During an infection, bacteria cause increased phosphorylation of the myosin regulatory light chain. Such phosphorylation regulates the function of epithelial tight junction complexes, which have a role in maintenance of barrier function, cell polarity as well as intercellular adhesion. Disruption of tight junction is associated with a carcinogenesis process. Wroblewski *et al*. showed that *H. pylori* urease may be connected with gastric cancer by causing damage to tight junctions.

Lately, a mechanism of activating blood platelets by bacterial urease has been described. Wassermann *et al*.showed that *H. pylori* enzyme stimulates this process through a lipoxygenase-mediated pathway. Such properties may have a role not only in gastrointestinal, but also cardiovascular diseases.

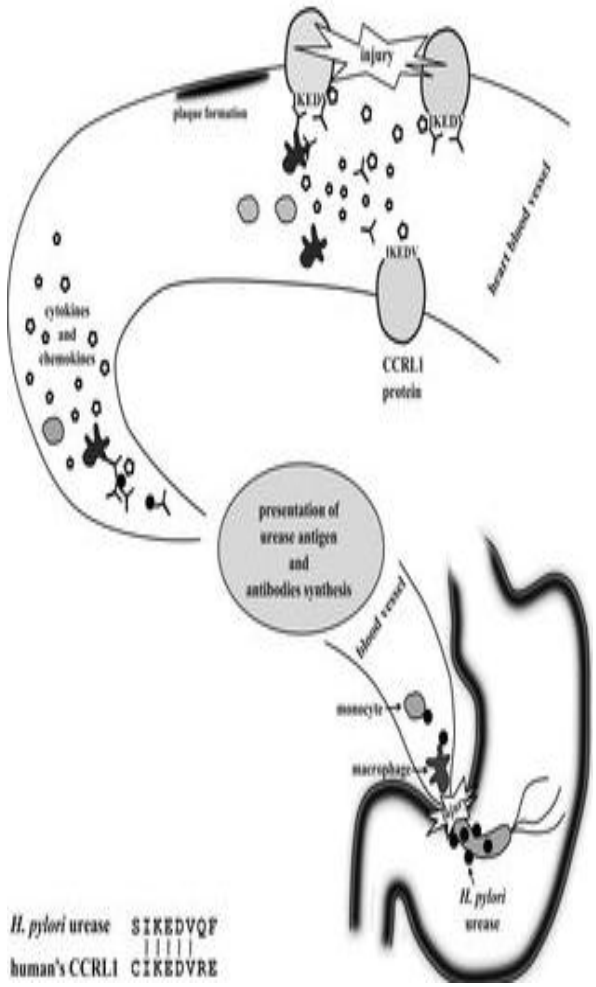
Prensence of anti-urease antibodies in sera of patients with long lasting disease: Bacterial ureases are considered to be one of the major antigens in several human diseases. Hirota *et al.* showed that this protein is immunogenic. In the flap region of enzyme, the ELR motive associated with immunogenic antigens is present. In long-lasting diseases caused by ureolytic bacteria, urease may stimulate generation of antibodies.

Infections of *H. pylori* are mostly chronic and, in many cases, lifelong. During a infection, an elevated level of immunoglobulins (secretory as well as circulating) was observed. Different classes of antibodies were noted: in

the stomach - IgA and IgM, in serum - IgG and IgA. IgG immunoglobulins remain even for a few months after bacterium eradication. Urease from this bacterium is one of the major immunodominant antigens. It is considered a vaccine in preventing *H. pylori* infections. In animal model, vaccination with *H. pylori* urease provides a significant and long term protection against a bacterial infection. In humans, oral administration of such a vaccine resulted in a strong immune response with minimal side effects.

The presence of anti-urease antibodies in *H. pylori* seropositive individuals is correlated with age and living in highly developed regions. Leal-Herrera, in the investigations performed on a population in Mexico, revealed that the percentage of infected individuals increases with age. The presence of anti-urease IgG antibodies in serum rises from less than 20% in a group of individuals below 10 years old to more than 50% - in a group over 40 years old. Occurrence of anti-urease antibodies was correlated with disease severity. In patients with superficial gastritis, a low level of IgG, but relatively high of IgA immunoglobulins was observed. Strong IgG reaction dominated in quiescent atrophic gastritis individuals, whereas in patients with active atrophic gastritis, reaction of IgG as well as IgA was very strong.

Nurgalieva *et al.* observed the presence of IgM antibodies, putatively recognizing a small subunit (UreA) of *H. pylori* urease in 94% of *H. pylori*-infected volunteers. The larger subunit - UreB seems to be less immunogenic. About 44% of the investigated individuals showed a positive reaction. However, Burnie and Al-Dughaym showed that UreB subunit of *H. pylori* urease has more epitopes recognized by antibodies than UreA. The level of IgG antibodies recognizing some of those epitopes was comparable with the commercial test. Also in Arabski *et al.* study, where levels of IgG antibodies were detected, the presence of antibodies recognizing *H. pylori* UreB urease subunit was found in almost all infected individuals. They were observed even in 70% of *H. pylori* negative sera. A much more interesting observation was a correlation between atherosclerosis and the presence of anti-urease antibodies. In the investigated sera there was a significant relationship between the level of antibodies bound to 8-mer synthetic peptide (which corresponds to UreB minimal flap epitope of *H. pylori* urease) and occurrence of atherosclerosis , an inflammatory disease leading to an atheromatosus plaque in blood vessels lumen. Earlier, Oshima *et al.* suggested that chronic *H. pylori* infections are connected with inflammatory processes in vessels. Investigations applying synthetic peptide corresponding to 8 amino acid sequence of flap fragment of *H. pylori* urease revealed a similarity between this peptide and human CCRL1 (CC chemokine receptor-like 1) protein, expressed mainly in the heart. Based on this observation, a hypothesis to explain a connection of *H. pylori* urease and atherosclerosis was formulated. According to it, urease may stimulate immune system reaction during a bacterial infection. Presentation of urease fragments to Th lymphocytes enables synthesis of antibodies. Next, antibodies against flap region of urease react with bacterial antigen. However, they may also recognize IKEDV motive in CCRL1 (due molecular mimicry) and cause an inflammatory process (Fig. **7**).



Molecular mimicry of flap fragment of *H. pylori* urease and CCRL1 and possible connection with atherosclerosis progress.

Rheumatoid arthritis (RA) is a classic long-lasting disease. It is an inflammatory condition leading to joint injury. During its progress, hyaline cartilages of joints as well as bones undergo atrophy. Etiology of RA is complex and, despite many years of investigations, still unclear. Apart from genetic background of RA occurrence, a role of infectious agents, like *P. mirabilis*, *Borrelia burgdorferi*, *Mycoplasma* sp., *M. tuberculosis*, *E. coli*, and *Porphyromonas gingivalis* as well as some viruses was discussed. Some of them are capable of urease synthesis. Among them, the most important is *P. mirabilis*. Wilson *et al.* revealed a connection of bacterial urease with disease progress. They showed a molecular mimicry between IRRET motive in *P. mirabilis* urease and human type XI collagen (LRREI sequence) present in hyaline cartilage.

The observed similarities concerned a sequence as well as a conformation fragments of both proteins. Simultaneously, the level of antibodies against *P. mirabilis* urease was significantly higher in comparison to healthy individuals as well as patients with ankylosing spondylitis - another autoimmune disease. According to Wilson *et al.* hypothesis, antibodies arising in reaction against bacterial urease function as autoantibodies and recognize also human protein (collagen). This leads to primary cytotoxic damage to hyaline cartilage. In the next step, in an injury site the presence of cytokines, vascular adhesion molecules and hydrolytic enzymes is observed. It causes inflammation, fibrosis and destruction of joints.

This hypothesis was confirmed in later studies. Konieczna *et al.* observed for RA patient's sera a significantly higher level of antibodies recognizing synthetic peptide corresponding to flap epitope of *P. mirabilis* urease. Surprisingly, they noted an elevated IgG level against peptides reflecting a sequence of flap regions from other organisms (bacteria and plant). The detected antibodies also had lower specificity. These antibodies recognized not only one defined antigen, but also antigens with a similar sequence, which was probably due to instability of the immune system.

A role of urease in stimulation of immune response of patients with immune disease was also revealed for other gram-negative bacteria. In 1993, it was showed that β subunit of *Y. enterocilitica* O:3 urease is arthritogenic for rats. A few years later, a high humoral response in patients with reactive arthritis triggered by *Y. enterocolitica* O:3 was noted. IgG reacting with 19 kDa urease subunit was observed in over 90%, and IgA in over 50 % of investigated sera.

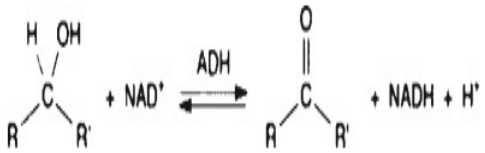
In chronic obstructive pulmonary disease (COPD) caused by nonencapsulated *H. influenza*e, urease is a target of human humoral response. In almost 39% of investigated sera, a significantly higher level of antibodies reacting with bacterial urease was observed.

Anti-urease antibodies are detected even in case of chronic zoonosis. In patients with diagnosed brucellosis, antibodies recognizing α *Brucella suis* urease subunit were detected.

In the investigations of antibodies generated as a response to infection, chemically defined synthetic peptides have a potent application. They are useful for epitope mapping as well as molecular mimicry studies.

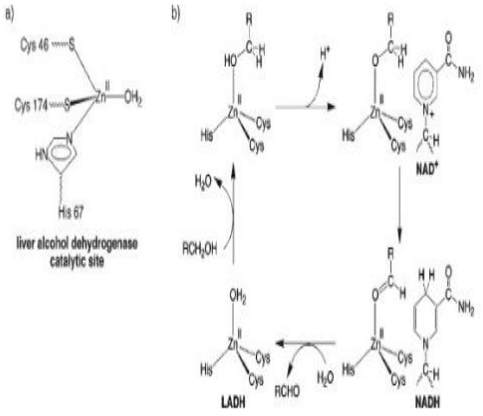
Alcohol Dehydrogenases

Alcohol dehydrogenases are a class of zinc enzymes, which catalyse the oxidation of primary and secondary alcohols to the corresponding aldehyde or ketone by the transfer of a hydride anion to NAD^+ with release of a proton:



The most extensively studied alcohol dehydrogenases are those of mammalian liver. They are dimeric proteins, with each subunit binding two Zn^{2+} ions, only one of which is catalytically active. This catalytic Zn^{2+} ion has distorted tetrahedral geometry, coordinated to one histidine and two cysteine residues. The non-catalytic zinc plays a structural role and is coordinated tetrahedrally to four cysteine residues.

The essential features of the catalytic cycle are summarized in. After binding of NAD^+ the water molecule is displaced from the zinc atom by the incoming alcohol substrate. Deprotonation of the coordinated alcohol yields a zinc alkoxide intermediate, which then undergoes hydride transfer to NAD^+ to give the zinc-bound aldehyde and NADH. A water molecule then displaces the aldehyde to regenerate the original catalytic zinc centre, and finally NADH is released to complete the catalytic cycle.



Thus, the role of zinc in the dehydrogenation reaction is to promote deprotonation of the alcohol, thereby enhancing hydride transfer from the zinc alkoxide intermediate. Conversely, in the reverse hydrogenation reaction, its role is to enhance the electrophilicity of the carbonyl carbon atom.

Tissue damage, metabolic derangements, and disease associated with ethanol metabolism

Tissue Damage

The direct actions of alcohol (e.g., disordering of membrane components and effects on signaling proteins) and the indirect effects resulting from ethanol metabolism described in the previous sections act in concert to induce tissue damage. In fact, ethanol metabolism often is considered to be the predominant

factor causing alcohol-associated tissue damage, particularly through the generation of ROS and oxidative stress in the tissues. ROS are generated during ethanol and acetaldehyde oxidation both by ADH/ALDH and by CYP2E1. The rate of ethanol and acetaldehyde oxidation by ADH and ALDH is determined by the rate with which the NADH generated can pass through the mitochondrial electron transport system. Because the mitochondrial electron transport system requires oxygen and generates ATP, the rate of NADH oxidation depends both on the cell's oxygen supply and on its demand for ATP. If either of these two factors is limited, electron transport activity is reduced. This has two effects: First, ethanol and acetaldehyde are inefficiently metabo-lized, and, second, electrons passing through the mitochondrial electron transport chain are "diverted" into forming harmful ROS, mainly superoxide (Hoek et al. 2002). Because ethanol metabolism by ADH and ALDH occurs primarily in the liver, any adverse effects associated with ethanol metabolism by these enzymes and associated ROS production primarily would affect that organ.

Effects on Fetal Development

Oxidative stress plays an important role in ethanol-induced damage to the developing fetus (Cohen-Kerem and Koren 2003). Low levels of CYP2E1 are found in prenatal brain (Brezezinki et al. 1999), suggesting that CYP2E1derived ROS could play a role in the development of alcohol-related birth defects, including fetal alcohol syndrome (FAS). Moreover, ROS produced during CYP2E1-mediated ethanol metabolism would likely be particularly harmful because the fetal brain shows only low levels of antioxidant enzyme activity compared with adult brain (Henderson et al. 1999). Researchers have studied whether administration of antioxidants, such as N-acetyl cysteine, SAMe, folic acid, and vitamin C, could improve cell survival during fetal ethanol exposure; however, these studies have yielded mixed results.

Impairment of Other Metabolic Processes

Chronic ethanol consumption and alcohol metabolism also may influence various other metabolic pathways, thereby contributing to metabolic disorders frequently found in alcoholics, such as fatty liver and excessive levels of lipids in the blood (i.e., hyperlipidemia), accumulation of lactic acid in the body fluids (i.e., lactic acidosis), excessive production of chemical compounds known as ketones in the body (i.e., ketosis), and elevated levels of uric acid in the blood (i.e., hyperuricemia).

The liver is most commonly affected by alcohol-induced damage. The first stage of liver damage following chronic alcohol consumption is the appearance of fatty liver, which is followed by inflammation, apoptosis, fibrosis, and finally cirrhosis. The development of fatty liver is induced by the shift in the redox state of the hepatocytes that results from ethanol metabolism by ADH. This shift in the redox state favors the accumulation of fatty acids, rather than their oxidation. In addition to these metabolic effects, chronic ethanol consumption contributes to the development of fatty liver by influencing the activities of several proteins that help regulate fatty acid synthesis and oxidation.

Chronic alcohol consumption also is associated with disturbances in the metabolism of sulfur-containing amino acids, leading to increased levels of the amino acids glutamate, aspartate, and homocysteine in alcoholic patients. These increases may have serious adverse effects. For example, homocysteine increases and modulates certain nerve signaling processes, particularly during alcohol withdrawal, and increases in homocysteine levels may possibly contribute to the alcoholism-associated tissue shrinkage (i.e., atrophy) observed in brain tissue (Bleich et al. 2004).

Clinical significance

Alcoholism

There have been studies showing that ADH may have an influence on the dependence on ethanol metabolism in alcoholics. Researchers have tentatively detected a few genes to be associated with alcoholism. If the variants of these genes encode slower metabolizing forms of ADH2 and ADH3, there is increased risk of alcoholism. The studies have found that mutations of ADH2 and ADH3 are related to alcoholism in Northeast Asian populations. However, research continues in order to identify the genes and their influence on alcoholism.

On the other hand, it seems that there have been mutations in ADH that have been naturally selected because they protect against alcoholism. It could be that they speed up the conversion of alcohol into acetaldehyde causing drinkers to feel unwell.

Drug dependence

Drug dependence is another problem associated with ADH, which researchers think might be linked to alcoholism. One particular study suggests that drug dependence has seven ADH genes associated with it.

These results may lead to treatments that target these specific genes. However, more research is necessary.

Poisoning

Fomepizole, a drug that inhibits alcohol dehydrogenase, can be used in the setting of acute methanol or ethylene glycol toxicity. This prevents the conversion of methanol to its toxic metabolites, formic acid and formaldehyde.

Structure and Function of Arginase

Introduction

Arginase was discovered in mammalian liver tissue by Kossel and Dakin in 1904. The enzyme belongs to family of ureohydrolases. These proteins serve an important role in the metabolism of arginine, the urea cycle, and other similar pathways. Arginase specifically assists in the removal of ammonia from the body. Arginase is crucial to life in all species and thus

catastrophic problems can occur if the enzymatic protein is deficient within the body.

Isozymes

Arginase is the only enzyme within the urea cycle that is found in two different isozymes. Isozymes are enzymes that differ in their amino acid sequence but still have the ability to catalyze the same reactions. Isozymes often express different regulatory properties. The existence of isozymes permits the refining of metabolism to meet the particular needs of a given tissue. In many cases, they are coded for by homologous genes that have evolved over time. There are two types of arginase found in the body, type I and type II. The type of isozymes is determined by the encoding genes. Arginase I is an enzymatic protein that has a total size of 34,700 Daltons, and is expressed in erythrocytes in humans and higher primates. The second isoform, arginase II, is a mitochondrial protein with a total size of 36,100 Daltons and is expressed in extrahepatic tissues like the small intestine, kidney, brain, monocytes, and macrophages.

Structure

A common feature of all arginases studied so far, whether eukaryotic or prokaryotic, is the requirement of cations with a positive two charge for activity. Manganese^{2+} is, by far, the most common activator of arginase, although the divalent cation requirements for some forms of the protein have been reportedly satisfied by cobalt, nickel, and iron [*check the "Manganese" box to the left to view the location of these ions*]. Experiments have shown that fully manganese-activated arginase contains two manganese (II) ions per subunit and that these ions form electron paramagnetic resonance (EPR) spin-coupled binuclear centers. EPR is a technique that has been proven to be beneficial to examining and understanding chemicals that have one or more unpaired electrons. EPR is comparable to the processes of nuclear magnetic resonance (NMR), except it analyzes the spin of the unpaired electrons instead of the spin of atomic nuclei. Detailed analyses of the temperature dependence of the manganese (II) EPR properties indicate a separation of 3.36–3.57 Å between manganese ions in the native enzyme.

These include type I arginase found in the liver of rats, a form of the recombinant human kidney type II arginase, and the arginase from the extreme thermophile *Bacillus caldovelox*. The form of arginase found in the liver is a 105 kDa homotrimer and each 35 kDa subunit contains a binuclear manganese (II) center that is a crucial aspect for the correct catalytic activity of arginase. The polypeptide fold of human arginase II is structurally identical to the alpha and beta folds of rat arginase I and the hexameric arginase from *Bacillus caldovelox*. "The structure of the binuclear manganese (II) cluster of human arginase II is nearly identical to that of rat arginase I, a finding that is not surprising considering that all metal ligands are conserved between the 2 sequences".

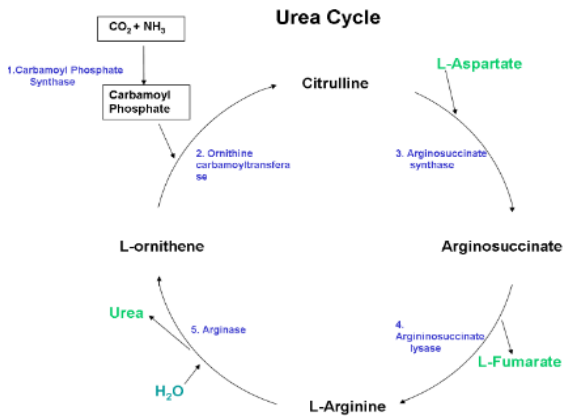
Arginase has two subunits. The fold of each subunit consists of a parallel, eight stranded ß-sheet bordered on either side by multiple α-helices The binuclear manganese center may be found at the bottom of an approximately 15 Å-deep active site cleft. The manganese ions are separated by 3.3 Å. This value may be verified by EPR measurements. Mn_A, is found deeper in the active site. It has square pyramidal coordination. The His 101 and Asp 128 are known to be terminal ligands while the Asp 124, Asp 232 and hydroxide ions are deemed bridging ligands. Mn_B coordinates with the "distorted octahedral geometry" by His 126 and Asp 234, terminal ligands, and Asp 124, Asp 232 and hydroxide ion, bridging ligands. In addition, a metal-bridging hydroxide can donate a hydrogen bond to the noncoordinating oxygen of Asp 128.

Conservation of Residues

Members of the arginase family commonly are approximately three hundred amino acids in length and have two manganese ions in the proteins active site *(2)*. There are twenty residues that are commonly conserved within the sequences of the arginase family These include five proline residues (found at locations 14, 28, 144, 238, and 280 in the amino acid chain) and five glycine residues (found at locations 23, 142, 178, 245, and 250 in the amino acid chain). Conserved residues often initiate or terminate a strand or support large loop regions. For example, Gly-23 initiates the first α-helix of all arginase proteins. Gly-178 is the last residue on β-5 and is found prior to a loop containing Asp-181. While Asp-181 is not well conserved, the residue and its substitutes commonly interact with the α-amino group of the arginine substrate in the urea cycle. This exemplifies the fact that a protein's structure is more commonly conserved than protein's sequence. The most highly conserved residue is His-141. This residue is thought to serve an important catalytic function in arginase as a "proton shuttle" because it has been found in rat liver approximately 4.2 Å from the solvent molecule that connects the manganese ions.

Function of Arginase

Arginase is the final step in the essential pathway known as the Krebs-Henseleit Ornithine Cycle or the Urea Cycle. Proper arginase function is critical to ensure dispersion of ammonia from the animal's body. In the muscle, amino acids may be metabolized into glucose, utilized in fatty acid synthesis, or catabolized to generate adenosine triphosphate (ATP) for energy. Excess amino acids are readily converted to glutamate in the muscle cells. Glutamate is transformed into alanine by alanine aminotranferase. In this form, the molecule can readily travel into the liver for further processing in this convoluted cycle. Amino acids can enter the cycle for urea synthesis via one of two pathways: transamination or transdeamination



Regulation of Arginase Levels

Arginase is constitutive in the body. Its activity is typically related to the level of arginase proteins. The number of arginase proteins is determined by the transcription of the arginase genes. However, there are several agents that can promote or suppress its activity in the body. This paper will briefly discuss some of the findings of arginase regulation from the review of immunohistochemical studies. The primary focus will be regulating arginase in vascular smooth muscle tissues and endothelial cells.

Arginase levels in vascular smooth muscle cells may be influenced by interleukin-4 and interleukin-13, transforming growth factor- β, lysophosphatidycholine, and mechanical strain. Expression of arginase in endothelial cells may be controlled by lipopolysaccharides, tumor necrosis factor- α, or a combination of the two. Levels may also be induced by thrombin, high glucose concentrations, oxidized low-density lipoprotein, or hydrogen peroxide. There are few suppressants of arginase in endothelial cells. A few plant compounds have been found to suppress arginase levels and prevent induction into the endothelial cells. These include genistein, cocoa flavanols, and simvastatin.

Consequences of High Concentration of Arginase in the Body

Imbalances of arginase levels in the body have been found to consequently induce vascular disease, pulmonary disease, infectious disease, immune cell function, and cancer. Over-expression of arginase has been found to affect proteins, nitric oxide, urea, and ornithine. concisely illustrates this information.

Effects on Proteins

If the level of arginase far exceeds that of arginine, nitric oxide synthesis could be reduced and thus nitric oxide synthase could be promoted *(14)*. Nitric oxide synthase catalyzes the oxidation of the amidine nitrogen in arginine and serves as a vasodilator in the body. This could lead to pulmonary hypertension. In addition, this could trigger an increase or decrease in the expression of proteins such as the cationic amino acid transporter and the isoform of nitric oxide synthase known as iNOS. The cationic amino acid transporter promotes the uptake of arginine in the body. If the level of arginine is too high in the body then one could suffer from hyperargininemia. Hyperargininemia is an autosomal recessive defect in the arginase gene. Symptoms of this defect generally do not appear until after the first few years of life and include elevated arginine levels in the blood and cerebrospinal fluid. Additionally, arginine may also begin to compete with lysine and cysteine in the urinary amino acid sequence for reabsorption in the renal tubule. If the level is too low in relation to the amount of arginase present the body is prone to pulmonary hypertension. iNOS can readily react with oxygen and superoxide and create peroxynitrite. Peroxynitrite reactions can often lead to cellular damage of cytotoxicity. If iNOS is absent in the body, the individual is often susceptible to infections.

Effects on Nitric Oxide

In endothelial cells, an over-expression of either arginase I or arginase II can reduce nitric oxide synthesis. Nitric oxide is physiologically important in the body. It serves as a vasodilator and is important in blood pressure regulation. Nitric oxide influences or serves a role in penile erection, neurotransmitters in the brain, the peripheral autonomic nervous system, long-term potentiation, neurotoxicity, muscle relaxation, and the primitive immune system. It has been found to inhibit adhesion, activation, and aggregation of platelets. Low levels of nitric oxide can induce pylorospasm in infantile hypertrophic pyloric stenosis *(3)*.

Effects on Urea

Urea can inhibit the amount of nitric oxide that can be produced by activated pulmonary artery endothelial cells. In addition, urea can reduce arginase's K_m for arginine and activate the arginase. High levels of urea in the bloodstream are associated with pre-eclampsia and increase the likelihood that urea-dependent activation of arginase can cause pulmonary hypertension. High concentrations of urea commonly lead to hyperammonemia, increased levels of ammonia in the body, and to often the consequences are fatal.

Consequences of Low Concentrations of Arginase in the Body

An arginase deficiency is typically present from birth and can be life-threatening. The gene which controls the prevalence of arginase in the body is ARG1. The absence of this gene leads to slow growth during early childhood (typically between birth and three years of age), the development of spasticity, limited cognitive development, and episodic hyperammonemia. Hypoarginemia may be controlled by the utilization of pharmacologic agents such as sodium benzoate or sodium phenylbutyrate to remove excess nitrogen from the body and reduce plasma ammonia concentrations. Additionally, diets may be fed that are high in calories from carbohydrates and fats to reduce catabolism and the amount of excess nitrogen in the body, Lastly, physiological stabilization via intravenous fluids and

cardiac pressors may be required to prevent over-hydration and cerebral edema. If not controlled arginase deficiencies may progress to include “severe spasticity, loss of ambulation, complete loss of bowel and bladder control, and severe intellectual disability”. Additionally, seizures occur frequently among individuals with arginase deficiencies.

CONCLUSION

Arginase is critical because the deficiency can lead to devastating problems and even death. Progress in the study of arginase has evolved greatly with the assistance of new technology since its discovery in 1904. Arginase is used primarily in the urea cycle to catalyze the reaction of converting arginine and water into ornithine and urea. This reaction is essential for the removal of ammonia from the animal’s body.

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