



COMPUTATIONAL APPROACH IN THE TREATMENT OF AORTA MEDIAL AMYLOIDOSIS (AMA) BY IN SILICO ANALYSIS AND DOCKING STUDIES OF LACTADHERIN

P. Sessa Charan^{1*} and P. Sri Satya Sindhu²

¹Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh.

²Vignan University, Guntur, Andhra Pradesh.

***Corresponding Author: P. Sessa Charan**

Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh.

Article Received on 29/11/2017

Article Revised on 19/12/2017

Article Accepted on 09/01/2018

ABSTRACT

Lactadherin is a protein that is produced in our body helps in apoptosis of cells and also protection against disease causing microorganisms. Due to unknown reasons it forms amyloid mainly in persons aged above 55. This leads to a disease called Aorta Medial Amyloidosis. The characteristics of disease include artery stiffening. There is no cure for this disease and the treatment includes chemotherapy. Thus in quest of new drugs and treatment methods, through computational approaches, we aimed to exploit the traditional knowledge of India, the Ayurveda which is treatment using herbal extracts. Literature search revealed that lactadherin is transformed into medin, the amyloid. Further investigation revealed that there is no experimental structure available for the protein in PDB database. So through homology modelling, the 3D structure of the protein was predicted, model validated and used in docking studies with market drugs and also herbal compounds to predict the best drug that has good interaction with the protein based on their binding energy. The primary, secondary structure analysis, functional analysis of the protein was also carried out. It was found that the protein is alkaline in nature and is extracellular. Our results clearly showed that the herbal compounds have greater negative binding energies than the chemical drugs. Hops extract from *Humulus lupulus* have the maximum negative binding energy and thus has good interaction with lactadherin. This can be subjected to further validation using in vitro studies.

KEYWORDS: Molecular docking, Aorta Medial Amyloidosis, Lactadherin, Medin, Homology modelling, Ayurveda, Chemotherapy.

INTRODUCTION

Lactadherin is a membrane glycoprotein expressed in Breast epithelial cells.^[1] The smooth muscle cells of medial layers of aorta produces it. The gene responsible for lactadherin is MFGE8. It involves in many phagocytosis processes of the body. In 2002, Hanayama et al discovered lactadherin as a factor which opsonises apoptotic cells for their clearance by phagocytes.^[2] Apoptotic cells show up phosphatidylserine on their surfaces where lactadherin binds with their C2 domain. This triggers the macrophages and interaction with RGD motif of the lactadherin takes place via integrins. Thus lactadherin functions as a bridge between macrophages and apoptic cells.^[2] It is also known to have other functions in protecting against microbes, coagulation, gonadogenesis and fertilisation. It can function as a phospholipid blocking anti-coagulant.^[3] Due to vague reasons it is known to get fragmented and thus form amyloids. The ragged N-terminal of the lactadherin suggests that the fragments are generated due to enzymatic cleaving rather than alternate splicing.^[4]

These amyloid fragments are known as medin which consists of the middle part of C2 like domain constituting 207-364 positions of the lactadherin.^[4] A report from 1970 says that amyloid was found in aortas of almost every person aged above 55.^[5] Medin amyloid occurs throughout the whole medial layer, predominant deposits found at inner half facing intima.^[6,7] This deposition leads to a disease called Aorta medial amyloidosis (AMA). Amyloidosis are disorders characterised by the fragmentation of proteins having chemical features different from the parent protein. They are of two types systemic and localised based on their scope and location. So far Medin amyloid was observed in the walls of arteries and thus regarded as localised amyloidosis.^[8] About 30 proteins polymerise via soluble oligomeric intermediates to form insoluble amyloid which are associated with human diseases like Alzheimer's, Parkinson's and type II diabetes.^[9,10] But the problem is that the nature and cytotoxic mechanism of the amyloid was not elucidated well to guide the development of targeted therapies. There is no cure for amyloidosis

instead treatment includes reducing the precursor protein, inhibition of fibril formation and amyloid clearance. The effects of medin amyloid have been little studied and only a few reports were found regarding the consequences of the deposition. The effects of the disease include Thoracic aortic aneurysm and dissection, Temporal arteritis and Artery stiffening. Thus as the disease occurs mostly in aged people, it should be treated sensitively as high doses of medication or left unattended may be fatal. Doctors mostly recommend chemotherapy which suppresses the formation of amyloid protein. So there is an utmost necessity to formulate drugs targeting the amyloid. Hence our work treats lactadherin as a receptor to be targeted by the drugs for better treatment of disease. Through computational searches it was found that the protein has not been crystallized experimentally and thus 3D structure is not available. So we tried to establish its 3D structure through homology modelling which is an established approach for predicting 3D structures and the models were evaluated. We also used lactadherin as a receptor to targets drugs available in the market to treat the disease. Through literature search we found that amyloidosis has herbal targets.^[11] So we also took herbal compounds from various medicinal plants known to treat this disease. Molecular docking studies were carried out with 10 FDA approved drugs and 15 herbal compounds in order to find their efficiency to treat the disease. Structural and functional analysis of the protein were also carried out.

MATERIALS AND METHODS

Sequence retrieval

The protein sequence of lactadherin having accession number NP_005919.2 was retrieved from NCBI Website^[12] by the entrez query given “lactadherin homo sapiens”. This sequence was retrieved in FASTA format and used for further analysis.

Primary Structural Characterization

The protein's physiochemical properties including molecular weight, amino acid composition, theoretical isoelectric point, estimated half-life^[13] total number of positive and negative residues, extinction coefficient^[14], atomic composition, aliphatic index^[15], instability index^[16], grand average hydropathicity (GRAVY).^[17]

were computed using ExPASy ProtParam server.^[18]

Secondary Structural Characterization

The secondary structural features of lactadherin include Alpha Helix, Beta Sheet, Gamma turns were predicted using Self Optimized Prediction Method from alignment (SOPMA).^[19,20]

Tertiary Structure Prediction and Evaluation

Through literature search it was found that the protein lactadherin has no structure present in PDB, the protein structural database. So it was modelled using homology modelling technique using Swiss Model Workspace.^[21,22] The results include selected templates, sequence identity and model quality estimation based on QMEAN4 score and Z score.^[23]

The predicted models were validated using PROCHECK^[24], VERIFY3D^[25], RAMPAGE^[26], ERRAT^[27] and PRO Q.^[28]

Functional analysis

The motif regions present in the protein were identified using motif scan server.^[29] CELLO v.2.5.^[30] was used to identify cellular localisation of the protein. The number of cysteine residues present in the protein was found using CYC_REC tool.^[31]

Drugs and Herbal compounds

Chemical drugs such as minocycline, clarithromycin, clofazimine, dapsone, ofloxacin, rifadin, merphalan, biacelin, pramlintide, adriamycin and herbal compounds from medicinal plants such as Onion(*Allium cepa* L), Neem(*Azadirachta Indica*), Aloe vera (*Aloe vera* L), Licorice(*Glycyrrhiza glabra*), Vasaka(*Justicia adhatoda*), Berberis spp, Psorelea (*Psorelea corylifolia*), Noni(*Morinda citrifolia* L), Garlic(*Allium sativum* L), Acacia, *Dalbergia retusa*, Chaulmoogra(*Hydnocarpus wightianus*) and Hops (*Humulus lupulus*) were taken as ligands to dock against the protein. Their chemical structures were obtained from PubChem^[32] in SDF format. These compounds were then converted into PDB format using Openbabel.^[33] These compounds were screened based on Lipinski Rule of Five.^[34]

Table 1: Chemical drugs used for docking studies.

Chemical drug	PubChem ID
Clarithromycin	84029
Clofazimine	2794
Dapsone	2955
Minocycline	5467543
Ofloxacin	4583
Rifadin	5381226
Merphalan	4053
Biacelin	5281605
Pramlintide	70691388
Adriamycin	31703

Table 2: Natural herbal compounds used for docking studies.

Plant name	Compound	Pubchem ID
<i>Humulus lupulus</i>	Hops Extract	6850842
<i>Berberis spp</i>	Berberine	2353
<i>Psorelea</i>	Psorelen	6199
<i>Chaumoorga</i>	Hydnocarpic acid	110680
<i>Dalbergia retusia</i>	Retusin	5481240
Acacia	Isoquercetin	5280804
<i>Azadirachta Indica</i>	Salannin	6437066
<i>Allium sativum L</i>	Allicin	65036
<i>Allium cepa L</i>	Allin	87310
<i>Morinda citrifolia L</i>	Glucuronic acid	92283
<i>Adhatoda vasica L</i>	Daucosterol	5742590
	Vasicine	667496
<i>Aloe vera L</i>	Aloin	12305761
	Emodin	3220
<i>Glycyrrhiza glabra</i>	Licoricidine	480865

Molecular docking

The interaction between drugs and proteins were analysed using Hex 8.0.0 Cuda.^[35] The protein was taken as a receptor and the chemical and herbal compounds were taken as ligands. Based on their binding energy, electrostatic and vanderwal interactions, post screening analysis was carried out.

RESULTS

Physiochemical characterisation

Using expasy protparam tool the physiochemical characteristics were computed and given in table 3. The computed instability index was 31.83 indicating that the protein is stable in nature. The isoelectric point of lactadherin was found to be 8.47 which suggests that the given protein sequence was alkaline in nature. The aliphatic index of the protein was 83.67 denoting the relative volume of a protein that is occupied by aliphatic side chains.^[14] The GRAVY value calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence^[16] was -0.244. This values shows its superior interactions with water. The estimated half-life was 30 hours.

Table 3: Physiochemical characteristics computed using expasy protparam server.

Characteristics	Value
Protein	Lactadherin
Sequence length	387
Molecular weight	43104.95
Extension coefficient	2.001
Isoelectric point	8.47
Instability index	31.83
Aliphatic index	83.67
Estimated half life	30 hours
GRAVY	-0.244
-R	31
+R	36

Secondary structure characterisation

Using SOPMA tool the secondary structural features of the protein were computed. The predictions were given in table 4. It was found that the protein has 25.84% of Alpha helix (100), 24.81% of Beta sheets (96) and 8.53% of gamma turns (33). This shows that the protein has a dominant alpha helix structure which says that the protein is hydrophilic in nature which may help in further drug development process.

Table 4: Secondary structure elements computed using SOPMA tool.

Characteristics	Value
Protein	Lactadherin
Alpha helix	25.84%
Beta sheet	24.81%
Gamma turns	8.53%
Disulphide bridges	-

Functional analysis

Through CELLO Server it was found that the protein is extracellular in location. Motif scan server predicted that the protein contains N-myristoylation site maximum number of times. It also has an N-glycosylation site, Casein kinase II phosphorylation site and Protein kinase C phosphorylation site.

Table 5: Motif regions present in the protein sequence calculated using motif scan server.

Motif Information	No. of sites	Amino acid residues
N-glycosylation site	4	238-241,325-328,329-332,350-353
Casein kinase II phosphorylation site	4	120-123,198-201,240-243,295-298
N-myristoylation site	8	12-17,75-80,112-117,224-229,235-240,273-278,281-286,346-351
Protein kinase C phosphorylation site	7	90-92,217-219,251-253,295-297,318-320,340-342,352-354

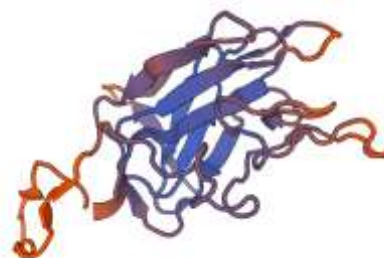
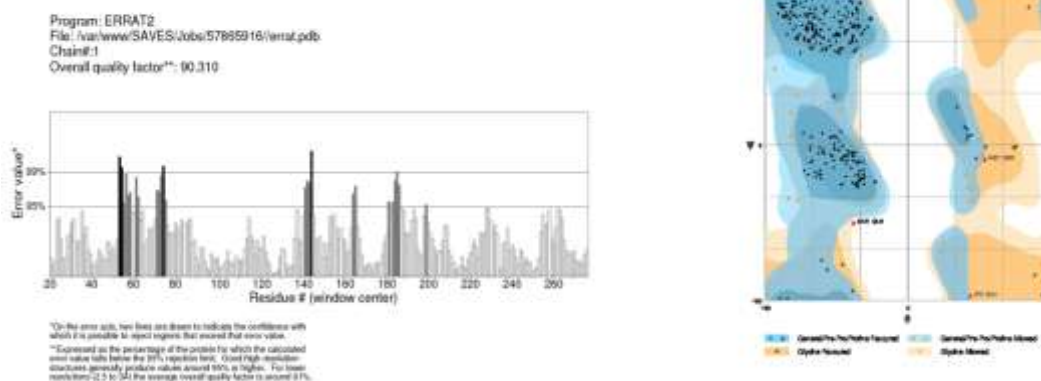
Homology modelling of Lactadherin

As there is no experimental data available for the structure of lactadherin in PDB database, it was build using swissmodel workspace. The protein sequence was retrieved from NCBI website and used for model

building. Models were build using templates and the best one was selected based on its QMEAN4 score and also Z Score (Fig 1). The model was further validated using different servers (table 6) which concluded that the model was of good quality.

Table 6: Validation parameters calculated using different servers.

Server	Value(%)
PROCHECK	90.6
VERIFY3D	91
ERRAT	90.31
PRO Q	LG score-3.340
	MaxSub-0.214
RAMPAGE (RFR)	91.8
Note: RFR-No. of residues in favourable region	

**Figure 1: Predicted 3D structure of Lactadherin.****Figure 2: ERRAT plot and Ramachandran plot for predicted 3D structure of Lactadherin.**

Molecular docking studies

Hex software was used to carry out docking studies. A total of 10 chemical drugs and 15 herbal compounds were made to interact with the protein. The receptor ligand interactions were measured in binding energy. The drug pramlintide showed the best interaction with the protein having binding energy -310.42. Then we moved on to the herbal compounds in which Hops extract from *Humulus lupulus* showed the best interaction with the protein having binding energy -394.94. So this showed that the Hops extract-lactadherin complex is more stable. The result can be validated in vitro using the herbal extract. The top 5 chemical compounds having highest negative binding energy were given in table 7.

Table 7: Top five compounds having highest negative binding energy.

Chemical compound	Binding energy
Hops extract	-394.94
Daucosterol	-325.78
Pramlintide	-310.42
Salannin	-310.34
Rifadin	-296.52

CONCLUSION

Lactadherin even though plays a vital role in defence and maintenance of the body, is fatal when form amyloid. Thus the disease should not be ignored and treated wisely. AMA treatment including high dosage of drugs is not advisable and so Ayurveda, the Indian traditional medicine should be considered which is safer and effective than the modern treatment. In our study taking

lactadherin as a receptor and both chemical drugs, herbal compounds as ligands, we proved that the herbal compounds have better interactions with the protein than the chemical drugs. Our results showed that Hops extract has a good fit with the protein lactadherin and thus can be used in the treatment of AMA. As Insilco approaches only ease the drug discovery process, the herbal extract is advisable to use in treatment of disease only after proper validation in vitro and in vivo.

ACKNOWLEDGEMENT

The authors acknowledge Mr. Nikhil for providing software to conduct docking studies.

REFERENCES

1. Newburg DS, Peterson JA, Ruiz-Palacios GM, Matson DO, Morrow AL, Shults J, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet*, 1998; 351: 1160–1164.
2. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S: Identification of a factor that links apoptotic cells to phagocytes. *Nature*, 2002; 417: 182-187.
3. Shi J, Gilbert GE. Lactadherin inhibits enzyme complexes of blood coagulation by competing for phospholipid binding sites. *Blood*, 2003; 101: 2628–2636.
4. Häggqvist B, Näslund J, Sletten K, Westermark GT, Mucchiano G, Tjernberg LO, Nordstedt C, Engström U, Westermark P: Medin: an integral fragment of aortic smooth muscle cell-produced lactadherin forms the most common human amyloid. *Proc Natl Acad Sci U S A*, 1999; 96: 8669-8674.
5. Schwartz P: Cardiovascular Amyloidosis. Amyloidosis, Cause and Manifestation of Senile Degeneration. Springfield, Charles C Thomas, 1970; pp 80.
6. Mucchiano G, Cornwell GG, 3rd, Westermark P: Senile aortic amyloid. Evidence for two distinct forms of localized deposits. *Am J Pathol*, 1992; 140: 871- 877.
7. Battaglia S, Trentini GP: [Aortic amyloidosis in adult life (author's transl)]. *Virchows Arch A Pathol Anat Histol*, 1978; 378: 153-159.
8. Westermark P, Benson MD, Buxbaum JN, Cohen AS, Frangione B, Ikeda S, Masters CL, Merlini G, Saraiva MJ, Sipe JD: A primer of amyloid nomenclature. *Amyloid*, 2007; 14: 179-183.
9. C.M. Dobson, Protein aggregation and its consequences for human disease, *Protein Pept. Lett.*, 2006; 13: 219–227.
10. D.J. Selkoe, Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases, *Nat. Cell Biol.*, 2004; 6: 1054–1061.
11. P Sessa Charan, Priyanka Narad: INSILICO ANALYSIS AND DOCKING STUDIES OF SEMENOGELIN 1 TARGETING SENILE SEMINAL VESICLE AMYLOIDOSIS (SSVA). *wjpmr*, 2017; 3(8): 182-186.
12. Lactadherin isoform a preproprotein [Homo sapiens] .https://www.ncbi.nlm.nih.gov/protein/NP_005919.2 .(Accessed 3/01/2018)
13. Gonda, D. K., Bachmair, A., Wunning, I., Tobias, J. W., Lane, W S. and Varshavsky, A., A Universality and structure of the N-end rule. *J. Biol. Chem*, 1989; 264: 16700–16712.
14. Gill, S. C. and Hippel, P. H. V. Calculation of Protein Extinction Coefficient from Amino Acid Sequence Data. *Analytical Biochem*, 1989; 182: 319-326.
15. Ikai, A. Thermostability and aliphatic index of globular proteins. *J. Biochem*, 1980; 88: 1895-1898.
16. Guruprasad, K., Reddy, B. V. B. and Pandit, M. W. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Eng*, 1990; 4: 55-161.
17. Kyte, J. and Doolittle, R. F. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol*, 1982; 157: 105-132.
18. Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.; Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press, 2005; 571-607.
19. Geourjon, C. and Deleage, G. SOPMA: a self-optimized method for protein secondary structure prediction. *Protein Eng.*, 1994; 7: 157-164.
20. Geourjon, C. and Deleage, G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Computer applications in the biosciences: CABIOS*, 1995; 11: 681-684.
21. Arnold, K., Bordoli, L., Kopp, J. and Schwede, T., The SWISS- MODEL workspace: a web-based environment for protein structure homology modeling. *Bioinformatics*, 2006; 22: 195-201.
22. Bordoli, L., Kiefer, F., Arnold, K., Benkert, P., Battey, J. and Schwede, T. Protein structure homology modeling using SWISS-MODEL workspace. *Nature Protocols*, 2009; 4: 1-13.
23. Benkert P, Biasini M, Schwede T (2011) Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, 27(3): 343–350.
24. Laskowski R A, MacArthur M W, Moss D S, Thornton J M. PROCHECK - a program to check the stereochemical quality of protein structures. *J. App. Cryst.*, 1993; 26: 283-291.
25. Lüthy, R., Bowie, J. U., & Eisenberg, D. Assessment of protein models with three-dimensional profiles. *Nature*, 1992; 356(6364): 83-85. doi:10.1038/356083a0.
26. Lovell S, Davis I, Arendall W III, de Bakker P, Word J, Prisant M, Richardson J, Richardson D. Structure validation by C alpha geometry: phi, psi and C beta deviation. *Protein Struct Funct Genet*, 2002; 50: 437–450.
27. Colovos CV, Yeates TO. Verification of protein

- structures: patterns of non bonded atomic interactions. *Protein Sci*, 1993; 11: 681–684.
28. Cristobal S, Zemla A, Fischer D, Rychlewski L, Elofsson A (2001) A study of quality measures for protein threading models. *BMC Bioinform*, 2: 5.
 29. Pagni M, Ioannidis V, Cerutti L, Zahn-Zabal M, Jongeneel CV, Hau J, Martinn O, Kuznetsov D, Falquet L. MyHits: improvements to an interactive resource for analyzing protein sequences. *Nucl Acid Res.*, 2007; 35: 433–437.
 30. Yu C, Chen Y, Lu C, Hwang J. Prediction of protein subcellular localization. *Protein Struct Funct Bioinform*, 2006; 64: 643–651.
 31. CYS_REC: The Program for Predicting SS-bonding States of Cysteines and disulphide bridges in Protein Sequences.
http://www.softberry.com/berry.phtml?topic=cys_rec&group=programs&subgroup=propt (accessed 03/01/2018).
 32. National Center for Biotechnology Information. PubChem Compound database.
<http://pubchem.ncbi.nlm.nih.gov/> (accessed 02/01/2018).
 33. O'Boyle N. M., Banck M., James A. C., Morley C., Vandermeersch T., Geoffrey R., Hutchison Open Babel: An open chemical toolbox. *Journal of Cheminformatics In Journal of Cheminformatics*, 2011; 3(1): 33-14.
 34. Lipinski, C. A (2004) Lead and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, 1(4): 337-341.
 35. Lüthy R., Bowie J.U., Eisenberg D., Assessment of protein models with three-dimensional profiles. *Nature*, 1992; 356(6364): 83-5.