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ELECTROPHORETIC PATTERNS OF PROTEINS IN THE TISUUE AND MUCUS EXTRACTS OF *ARION ATER* (BLACK SLUG)

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ABSTRACT

The present study was undertaken to analyze the proteins qualitatively in the tissue and mucus extracts of terrestrial slug *Arion ater* (Black slug). The patterns indicated that the tissue extraction has higher number of protein bands compared to the mucus. The patterns of protein bands observed in tissue and mucus extraction of *Arion ater* using Sodium Dodecyl Sulphate Poly Acryl amide Gel Electrophoresis (SDS-PAGE) indicated a distinct pattern of three protein bands and some additional bands, with low resolution in the tissue extract of *Arion ater*, where as two protein bands in the extract of mucus. The electrophoretogram revealed that both the patterns of tissue and mucus extract showed homology in protein bands with minor variations.

KEYWARDS: *Arion ater*, SDS-PAGE, electrophoretogram, mucus, homology.

INTRODUCTION

Histochemistry has been the springboard for the identification in molluscan tissues (hypobranchial body and salivary glands) of a number of biogenic amines, choline esters and active peptides. Among the biogenic amines 5-HT and related indolealkylamines, octopamine, tyramine and histamines. Among the choline esters murexine, dihydromurexine, senecioylcholine and acryloylcholine; among the peptides an array of compounds belonging to at least ten different peptide families. 5-HT, octopamine, tyramine and histamine are present. Alkaloids, saponins, sterols, poly phenols, flavonoids and sesquiterpene lactones are important common biomolecules present in the gastropods.

The bodies of land pulmonata such as snails and slugs are characterized by rich mucus which covers their surface. Apparently, the mucus may serve in preventing the moisture evaporation, in helping smooth movements and in protecting the body from mechanical injuries. In addition, some unknown biochemical function may be involved in the mucus, though nothing has been reported so far with this respect.[1] ***Glycoproteins (GP),*** physiologically active biomacromolecular structures, widespread in the animal world, are **heteroproteins** (proteins conjugated) structured from a carbohydrate (polysaccharide with fragments of N-acetyl hexosamine, different monosaccharides, and uronic acids), is called **mucopoly-saccharide** (immunopolysaccharide) as prostetic group and proteins proper, predominantly quantitatively. Depending on the nature of the linking, in most glycoproteins, there are three glycosidic bridges: (O) – glycosides, (C) – glycosides and (N) glycosides, respectively. Mucin glycoproteins are the major macromolecular constituents of epithelial mucus and have long been implicated in health and disease. Mucins historically are large, highly glycosylated, viscoelastic macromolecules that are difficult to isolate and purify mentioned fields.[2] Proteins are glycosylated mucin composition, over 90% are glycoproteins.[3]

MATERIAL AND METHODS

Animal materials chosen for the study

The Slugs were collected from fields of Komatipally village, Warangal,Telangana, India.The collected animals were identified by using standard manuals.

Exatraction and collection of Samples

The Mucus (5 ml) was collected from roughly 50 individuals by stimulating the surface of live slugs by small plastic syringe (5 ml). The samples were stored at -20°C in a deep freezer until analysis according to Sallam *et al*., After collecting the mucus whole body were crushed and weighed to the nearest milligram. The mucus as well as tissue extractions were homogenized (10%) in 0.01M Tris-Hcl buffer (pH 7.0) containing 0.1% sodium dodecyl sulphate (SDS) and 0.9% NaCl the extracts were centrifuged at room temperature (30±2^{o}C).

Experimental procedure for preparation of SDS-PAGE

The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS β-mercaptoethanol and bromophenol blue as the tracking dye. An aliquot of 0.1ml (5mg) of the tissue extract was loaded on to the separating gel directly. The electrode buffer 0.025M tris and 0.192M Glycine was used for Lamelli's method[4] whereas 0.074 M Tris, 0.1% SDS

adjusted to pH 7.8 with concentrated HcL. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin.

Staining Procedure and standardization of protein bands

A solvent containing 0.25% Coommassie brilliant blue in methanol, water, acetic acid (5:5:1) was used for staining the proteins separated on gel by using standard method.[4] The molecular weight standards used in comparing the variations noticed in the SDS-PAGE were low molecular weight protein standards (23 to 70 KDa) from the SIGMA-Chemical company from (USA).

RESULT AND DISCUSSION

The protein patterns of *Arion ater* observed in tissue and mucus extracts and their relative mobility (Rm) are presented in (Table.1 and Fig.1) respectively. The protein patterns observed on SDS-PAGE stained with Coomassie brilliant blue indicated distinct differences in the mobility of some bands of the extract of mucus and tissue. Comparison of the protein bands of the various regions with standard marker proteins revealed that the medium in the regions of slow moving zones "A' (mol weight 70KDa) and those with the fast moving zone "C" (mol. wt 28KDa, 23KDa) The patterns observed in the middle region "B" (mol.wt 55KDa , 35KDa) is less similar in mucus and tissue extract.

Table1: Rm values of tissue and mucus extract of *Arion ater* (Black slug) through SDS-PAGE.

S.No	Molecular marker standars	Tissue extract	Mucus
1	70	0.21	-
2	55	0.30	0.28
3	35	-	-
4	28	0.40	-
5	23	0.48	0.48

The electrophoretic patterns of tissue and mucus extraction on SDS-PAGE indicated a less number of protein bands in mucus with decrease in the intensity compared to tissue extract of *Arion ater* slug. In the slow moving zone "A' (mol.weight 70KDa) a protein band with Rm value 0.21 (mol.wt 70KDa) showed low intensity in tissue extract and it is not observed in mucus extract. A protein band with Rm value 0.30 and 0.28 (mol. wt 55KDa) were observed in extract of tissue and mucus respectively. The Rm value 0.40 (mol.wt 28) was observed in only tissue extract of *Arion ater* but it is not observed in extract of mucus. The Rm value 0.48

(mol.wt 23KDa) was observed in both tissue and mucus extract (Zone 'C"). The Rm value of protein band 0.40 in between the molecular weight (35KDa-28KDa) was completely disappeared in mucus extract.

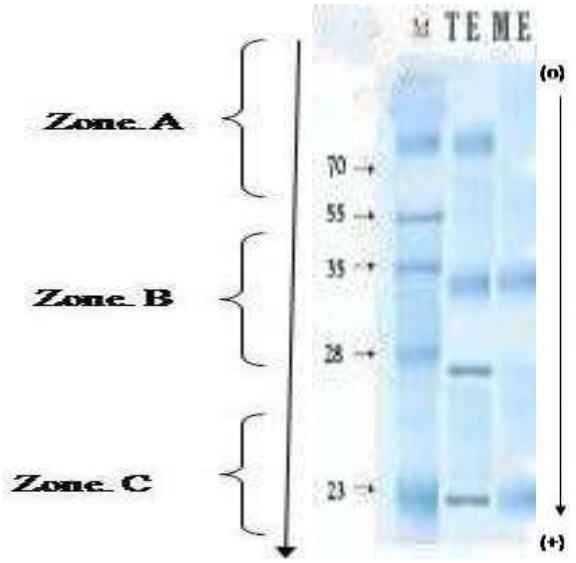

Fig. 1: SDS-PAGE Electrophoretic Patterns of Proteins of *Arion ater* tissue and mucus extract stained with Commassie brilliant blue

Right lane indicates (M) mol.weight standars (23KDa to 70KDa), 'A','B','C' zones TE=Tissue extract, ME=Mucus ectract: zone A=mol wt(70KDa), zone B(35KDa to 55KDa), zone 'C" (23KDa to28KDa) , + = indicates anode. ↓ =Direction of run.

DISCUSSION

The pattern of proteins observed in the tissue and mucus extract of slug on SDS-PAGE gel indicated a distinct of three protein bands and one additional band with weak staining on the gel in slow moving zone. Therefore, the protein patterns of tissue and mucus extraction of the slug exhibited some regions are nearest similarity (Fig.1).

The presence of protein bands with identical mobility in the tissue and mucus extract indicated the similarity of proteins secreted by mucus glands (venom).Various authors have reported that the Venoms are animal secretions predominantly injected into another animal for the purposes of predation or defense, and also have important biological applications with biomedical relevance.[5] In the present study SDS-PAGE analysis showed the presents of bioactive compounds responsible for antibacterial activities. The presence of antibacterial compounds in the oyster *Pteria chinersis* and bivalve *Perna viridis* have been reported using the various solvent extracts.[6, 7] As the molluscan resources are rich and varied in species to

species, there exist a great potential for the extraction of bioactive compounds of medicinal importance at a lower cost.

CONCLUSION

It can be concluded from our present investigation that both the patterns of tissue and mucus extract showed homology in protein bands with minor variations and the study demonstrates the effects of tissue and mucus extract on SDS- PAGE, characterization of the protein responsible for the bioactivity. Further purification and structural elucidation of compounds are required to confirm the designation of venoms and tissue in the proposed groups. This will greatly help utilize these compounds for the prosperity and well-being of human kind. Thus, the results of the present study indicate a very strong anti microbial activity of *Arion ater*. The study strongly suggests that use the commercially available and protein rich (bactericidal) in therapeutics for the development of novel antibiotics against multiple drug resistance (MDR) pathogenic microbes. Anti peptides could be utilized as a probing tool to investigate the pharmacological potential. These characteristics emphasize the need for isolation and molecular characterization of new active anti peptides in *Arion ater* in near future.

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REFERENCE

1. Simkiss K., Wilbur K. M., The molluscan epidermis and is secretion. Symp. Zool. Soc. Lond., 1977; 39: 35-76.
2. Rose M., Characterization of human tracheobronchial mucin glycoproteins. In: Methods in Enzymology, edited by V. Ginsburg. New York: Academic, 1989; 179: 3–17.
3. Uivaroșan I. G., Teză de doctorat, Studiul posibilităţilor de identificare a unor glicoproteine, cu potenţială activitate antimicrobiană, provenite din melci (Helix Pomatia si Helix Aspersa) crescuţi în partea de vest a României, 2011; Banat`s University of Agricultural Sciences and Veterinary Medicine.
4. Laemmli U. K. “Cleavage of structural proteins during the assembly of the head of bacteriophage T4,” Nature, 1970; 227: 680–685.

5. Twede, V. D., Miljanich, G., Olivera, B. M., Bulaj, G. Neuroprotective and cardioprotective conopeptides: An emerging class of drug leads. Curr. Opin. Drug Disc. 2009; 12(2): 231-239.
6. Chellaram, C., Gnanambal, K.M. and Edward, J.K.P. Antibacterial activity of the winged oyster, Pteria chinensis (Pterioidae: Pteridae). Indian J. Mar. Sci., 2004; 33: 369-372.
7. Li, C., Song, I., and Zhao, J., A review of advances in research on marine molluscan antimicrobial peptides and their potential application in aquaculture. Molluscan Res., 2009; 29: 17-26.
8. Brumfitt W, Hamilton-Miller JMT, Franklin I. Antibiotic activity of natural products: 1 Propolis. Microbios. 1990; 62: 19–22.
9. Lowry O.H, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biol Chem. 1951; 193: 265–275.
10. Heussen C, Dowdle EB. Electrophorectic analysis of plasminogen in polyacrylamide gels containing sodium dodecyl sulphate and copolymerized substrates. Anual Biochem. 1980; 102: 196–202.
11. Munro M.H.G, Blunt J.W, Dumdei E.J, Hickford S.J.H, Lill R.E, Li S. The discovery and development of marine compounds with pharmaceutical potential. J Biotechnol. 1999; 70: 15–25.
12. Tadesse M, Gulliksen B, Strom M.B, Styrvold O.B, Haug T. Screening for antibacterial and antifungal activities in marine benthic invertebrates from northern Norway. J Invertebr Pathol. 2008; 99: 286–293.
13. Elezabeth M.K.G, Chellaram C, Jamila P. Antimicrobial activity of reef associated gastropod, Trochus radiates. National Seminar on Ecosystem, Remdiation. 2003; 68.
14. Jayaseli AA, Prem Anand T, Murugan D. Antimicrobial activity of four bivalves from Gulf of Mannar, Phuket. Mar Biol Cent Spec Publ. 2001; 25(1): 215–217.
15. Chandran B, Rameskumar G, Ravichandren S. Antimicrobial activity from the gill extraction of Perna viridis (Linnaues, 1758) Glob J Biotech Biochem. 2009; 4(2): 88–92
16. Darabpour E, Motamedi H, Nejad SMS. Antimicrobial properties of Teucrium polium against some clinical pathogens. Asian Pac J Trop Med. 2010; 3(2): 124–127.
17. Kannan RR, Arumugam R, Anantharaman P. Antibacterial potential of three seagrasses against human pathogens. Asian Pac J Trop Med, 2010; 3(11): 890–893.
18. Bhattacharjee I, Chatterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of Argemone mexicana L. (Papaveraceae) Asian Pac J Trop Med, 2010; 3(7): 547–551.

19. Shenava A., 'Synthesis of Silver Nanoparticles by Chemical Reduction Method and Their Antifungal Activity. International Research Journal of Pharmacy 2013; 4(10): 111-113.
20. Hammarstrom, S. and K abat, E.A. (Purification and characterization of a blood group A reactive hemagglutinin from the snail Helix pomatia and a study of its combining site. Biochemistry 1969; 8: 2696–2705.
21. Hommay, G., Kienlen, J.C., Gertz, C.and Hill, A.Growth and reproduction of the slug Limax valentianus Férussac in experimental conditions.Journal of Molluscan Studies, 2001; 67: 191–207.
22. Zou, H., W. J. Henzel, X. Liu, A. Lutschg, and X. Wang. Apaf-1, a human protein homologous to Caenorhabditis elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1970; 90: 405–413.
23. Jallepalli, P. V., C. Lengauer, B. Vogelstein, and F. Bunz.. The Chk2 tumor suppressor is not required for p53 responses in human cancer cells. J. Biol. Chem. 2003; 278: 20475–20479.
24. Sallam A.A.A., El-Massry S.A. & Nasr I.N. Chemical analysis of mucus from certain land snails under Egyptian condi tions. *Archives of Phytopathology and Plant Protection* 2009; 42: 874–881.