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Research Article

PROTEIN PATTERNS OF PAROTOID GLAND EXTRACT AND ITS SECRETION OF *BUFO MELANOSTICTUS* (SCHNEIDER) THROUGH UREA-SDS-PAGE

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ABSTRACT

Objective: The present investigation has been undertaken to study the electrophoretic banding patterns of proteins in the parotoid gland extract and its secretion of common Indian toad *Bufo melanostictus.*

Methods: Urea Sodium dodecyl sulphate and polyacrylamide gel electrophoresis (Urea-SDS-PAGE) of parotoid gland extract and its secretion was separated through 12% Urea-SDS-PAGE using Silver nitrate staining.

Results: The patterns of protein bands indicated a distinct pattern of six protein bands with some other additional bands with weak staining were observed in the parotoid gland extract and four protein bands in parotoid gland secretion and one additional band with weak staining. The protein patterns were identified by using a marker relative molecular weight of approximately 14-200 KDa. Rm value of proteins bands were calculated accordingly.

Conclusion: The electrophoretogram revealed that both the protein patterns of parotoid gland extract and its secretion showed homology in protein bands with minor variations.

Keywords: Parotoid gland, *Bufo melanostictus*, Rm value, Urea-SDS-PAGE.

INTRODUCTION

Amphibians like toads are characterized by the presence of cutaneous glands spread over the skin. Basically two different types of glands developed in the amphibian skin i.e., i) mucus secreting glands generally associated to maintain the humidity and cutaneous respiration and to protect the skin from mechanical damages and prevent microbial settlement on the skin; these glands secrete glycoprotein rich material which plays an important role in defense mechanism. ii) Granular glands generally associated with chemical defense against predators and microbial infection [1, 2, and **3**]. The product secreted by such glands contain a wide variety of rich components like biogenic amines, bufo toxins, oligo peptides, proteins, guanidine derivatives, steroids and alkaloids in terms of pharmacological effects [1, 4-6]. The epidermal glands in Amphibians are more evolved and are alveolar glandular cells and open on to the surface of the skin through ducts. In toads these glandular cells form the parotoid glands located between eyes and tympanum [2, 7]. The venomous secretions of the parotoid glands of the *Bufo* species are known to contain several bioactive compounds [8] and were used by Chinese, Indian traditional medicinal practice and Japanese physicians for centuries as folk medicines like "kyushin" and "Chan Su" [5,9]. The granular secretions are known to be secreting a variety of compounds which are species specific [10, 11].

So far, there are few reports on the protein patterns of *B. melanostictus* through Urea-SDS-PAGE. The present investigation has been undertaken for the study of protein patterns of parotoid gland secretion and its extract of *Bufo melanostictus* (Schneider) through Urea-SDS-PAGE in order to understand their possible defense role against microbial infections.

MATERIALS AND METHODS

Animal materials chosen for study

The toads (7 to 10cm in length, weighing about 45-70 grams) were collected from the vicinity of Kakatiya University hostel buildings.

Extraction and Collection of Samples

The parotoid glands were gently pressed to release the secretions. The secretions were collected in ice-jacketed containers. After collecting the secretions, the gland was dissected out and were blotted free of blood clots and other adherents, tissues were weighed to the nearest milligram. The parotoid gland extract as well as its secretions 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 Urea 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 10M-Urea separating gel directly. The lower chamber electrode buffer 1M Tris and 0.2% SDS adjusted to pH 7.8 with concentrated H_2SO_4 was used for the Anderson *et al.,* 1983[12] and whereas upper chamber electrode buffer 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 was used for Lamelli's method [13]. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin.

Staining Procedure with silver nitrate for Urea gel electrophoresis

For silver staining, the gel was placed in a fixative (50% methanol, 12%acetic acid) for 1 h with gentle shaking. Thereafter, it was submerged into 50%ethanol twice for 7 min each and washed twice with distilled water, 10 min each. The gel was treated with 0.025% Hypo for 1 min, rinse the gel in distilled water for 3 times for 1 min. then stain with AgNO3 (200mg/100ml) containing 75µl formaldehyde/100ml for 20 min. rinse the gel in distilled water for 40 seconds. The gels were developed in 100 ml of sodium carbonate solution containing 50 µl formaldehyde solutions for 5 to 10 minutes. The color formation was stopped by gradual washing in 1 to 5% acetic acid and finally stored in 5% acetic acid.

The gels were analyzed by silver nitrate staining method described by Switzer *et al*., 1979 [14].The destaining was done by repeated washing in 1 to 5% acetic acid and finally stored in 5% acetic acid.

Standardization of protein bands

The molecular weight standards used in comparing the variations noticed in the Urea-SDS-PAGE were of molecular weight protein standards (14 to 200 KDa) from the Bio-Rad-Chemicals company from USA.

RESULTS

The electrophoretogram obtained from parotoid gland extract and its secretions of the protein patterns of *Bufo melanostictus* and their

relative mobilities are presented in Figure 1 and Table 1, respectively. The protein patterns observed on Urea SDS-PAGE stained with Silver nitrate stain indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. Protein band comparison of various regions with standard marker proteins revealed that the variation is higher in the fast moving zone "C" (mol.wt.31-14KDa) and those with middle region "B" (mol.wt.66-45KDa). The pattern observed in the slow moving zone "A" (mol.wt.200-97KDa) is more or less similar in the secretion and gland extract.

The electrophoretic patterns of proteins of parotoid gland extract and its secretion on Urea SDS-PAGE indicated a less number of protein bands in parotoid gland secretion with decrease in the intensity compared to parotoid gland extraction. In the slow moving zone "A" (mol.wt.200-97KDa) a protein band with Rm value 0.21(molecular weight 116KDa) showed low intensity in parotoid gland extract and not observed in parotoid gland secretion. A protein band with Rm value 0.28(molecular weight 97 KDa) was observed in both parotoid gland extract and its secretion. The Rm value 0.42 protein band was observed only in parotoid gland extract and the Rm value 0.45 protein band was observed only in parotoid gland secretion in between the molecular weight (66-45KDa).Protein bands with Rm values 0.54, 0.60 observed in parotoid gland extract and the Rm with 0.58 in parotoid gland secretion nearer to the molecular weight 45KDa in the middle region "B" (mol.wt. 66-45KDa). In the fast moving zone "C" (mol.wt.31-14KDa) the Rm value with 0.68 (molecular weight 31KDa) was observed in the parotoid gland extract which was disappeared in parotoid gland secretion. The Rm value 0.78 (molecular weight 22KDa) was observed in parotoid gland secretion and disappeared in gland extraction. The Rm value 0.85 (molecular weight 14KDa) was observed in both parotoid gland secretion and its extract (Zone .C).

DISCUSSION

The pattern of proteins observed in the parotoid gland extract and its secretion of toad observed on Urea-SDS gel indicated a distinct of six protein bands and some additional bands with weak staining on the gel in fast moving zone, whereas a distinct of four protein bands were observed in the parotoid gland secretion and one additional band with weak staining on the gel in slow moving zone. Therefore, the protein patterns of parotoid gland extract as well as the gland secretions of the toad exhibited some regions of similarity (Fig 1).

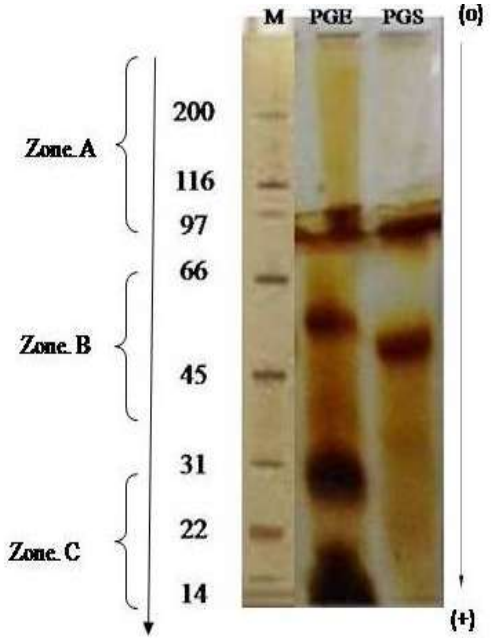

Fig. 1: Urea-SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus* Parotoid Gland Extract and its Secretion stained with Silver nitrate

Right lane indicates (M) mol. weight strands (200-14 KD.) 'A', 'B', 'C' zones, PGE = Paratoid gland Extract,PGS = Paratoid gland Secretion, M = Molecular weight standards (14 to 200 KD), Zone A = mol. wt. 200 KD,116 KD and 97 KD,Zone B = mol.wt.66 KD and 45 KD,Zone C = mol.wt. 31KD, 22 KD and14 KD; O = Origin, + = Anode, ↓ = Direction of run.

Table 1: Rm values of Parotoid gland extract and secretion of *Bufo melanostictus* (Schneider) through Urea-SDS-PAGE

Molecular Marker Standards	Parotoid Gland Extract	Parotoid Gland Secretion
0.14	-	-
0.21	0.21	-
0.28	0.28	0.28
0.35	-	-
-	0.42	-
-	-	0.45
0.50	-	-
-	0.54	-
-	-	0.58
-	0.60	-
0.68	0.68	-
0.78	-	0.78
0.85	0.85	0.85

The presence of protein bands with identical mobility in the secretions and gland extracts, indicated the similarity of proteins secreted probably by granular cells of epidermis. The secretary proteins exist as coiled filaments with in epidermal granular cells, [15].Various authors have reported that the alkaloids and steroids as toxic and anti-feeding agents, acting as a major chemical defense strategy against predators [16,17,18]. Such molecules act on the cardiovascular system, increasing the blood pressure and or raising the force of contraction of the heart [19, 20]. The presence of these arrays of proteins in *Bufo* parotoid gland secretions suggests a more complex role for these secretions than simply anti-predator defense. The peptides found in various species of toads and frogs which possess antimicrobial activities are of a much smaller molecular size range than encompassed by SDS-PAGE used here. For instance, the magainins found in skin secretions of *Xenopus* are typically of 21-26 amino acid residues in length [21].

CONCLUSION

In view of the above results, it can be concluded that the parotoid gland extract and its secretion contain several of these granular cells and when the gland is pressed the secretion are released in the form of sticky fibrillin material. When their electrophoretic patterns were observed on Urea-SDS gel revealed the presence of protein bands with identical mobility both in secretion and gland extract indicating homology and regions of similarity of cell lines and its secretion in *Bufo melanostictus*.

The present investigation reports that the analysis of protein patterns of *Bufo melanostictus* on Urea-SDS gel, inspite of some minor differences in the total protein concentration and relative concentration within the same sample, would lead to the conclusion that the secretions are very similar among themselves in *Bufo melanostictus.*

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