ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Electrophoretic patterns of proteins in the secretion of Parotoid gland and its extract in B*ufo melanostictus* (Schneider)

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Manuscript Info

Manuscript History:

Received: 15 July 2013
Final Accepted: 23 July 2013
Published Online: August 2013

Key words:
Protein patterns,
Bufo melanostictus,
Electrophoresis.

Abstract

The present study was carried out to analyze qualitatively the Electrophoretic patterns of proteins in Parotoid gland extract and its secretion in terrestrial toad *Bufo melanostictus* (Indian toad). The patterns indicated that the gland extraction has higher number of protein bands compared to the gland secretion. The patterns of protein bands observed in the parotoid gland extraction of *B. melanostictus* using Sodium Dodecyl Sulphate Poly Acryl amide Gel Electrophoresis (SDS-PAGE) indicated a distinct pattern of four protein bands and some additional bands, with poor resolution, where as two protein bands in the parotoid gland secretion. The electrophoretogram revealed that both the patterns of parotoid gland extract and its secretion showed homology in protein bands with minor variations.

Introduction

In lower vertebrates, the integument secretes slimy mucus, which is constantly replenished by the glandular cells present in the skin. In amphibians these cells are organized into two different glandular structures i.e., i) mucus secreting glands that help to keep the skin moist and slippery and protect skin from the mechanical damages and prevent microbial settlement on the skin. These glands secrete glycoprotein rich material which plays a part in cutaneous respiration and defense and ii) granular glands generally associated with chemical defense and against predators and microbial infection [1]. These gland secretions contain rich components like biogenic amines, bufotoxins, oligopeptides, proteins, guanidine derivatives, steroids and alkaloids in terms of pharmacological effects [1, 2-5]. 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, they form the parotoid glands located between eyes and tympanum [6]. The venomous secretions of the parotoid glands of the *Bufo* species are known to contain several bioactive compounds [7] and were used by Chinese and Japanese physicians for centuries as folk medicines [3]. The granular secretions are known to be secreting a variety of compounds which are species specific [8, 9]. The present investigations on Electrophoretic study of protein patterns of the parotoid gland extract and its secretions of the common Indian toad *Bufo melanostictus* were analyzed. This report reveals that complex array of proteins are present in extracts and secretions.

Material and Methods

Animal materials chosen for study

The toads (7cm 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 and gland as well as 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 centrifuge**d** 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 [10] 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.

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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 [10]. The molecular weight standards used in comparing the variations noticed in the SDS-PAGE were low molecular weight protein standards (14 to 66 KDa) from the SIGMA-Chemical company from (USA).

Result and Discussion

The protein patterns of *Bufo melanostictus* observed in parotoid gland extract and its secretions and their relative mobility (Rm) are presented in (Fig.1 and Table 1) respectively. The protein patterns observed on SDS-PAGE stained with Coommassie brilliant blue indicated distinct differences in the mobility of some bands of the parotoid gland extract and its secretion. Comparison of the protein bands of various regions with standard marker proteins revealed that the variation is higher in the regions of slow moving zones " A" (mol wt.66KDa) and those with fast moving zones "C" (mol.wt. 24KDa,14.2KDa). The pattern obtained in the middle region "B" (mol.Wt.45KDa, 36KDa) is more (or) less similar in secretion and gland extract.

The electrophoretogram obtained reveals that there is a decrease in the intensity of protein bands of parotoid gland secretion compared to protein bands of parotoid gland extraction. A protein band with Rm value 0.11 (nearer to molecular weight 66 KDa) showed decrease in the intensity in parotoid gland secretion whereas high intensity in parotoid gland extraction. The Rm values of protein bands 0.12, 0.21, 0.25 in between the molecular weight 66 KDa-45 KDa completely disappeared in parotoid gland secretion (Zone. A) in slow moving zone compared to parotoid gland extraction. The Rm value of protein bands 0.31, 0.50 and 0.52 in between the molecular weight of 45 KDa-35 KDa were completely absent in parotoid gland secretion (Zone. B) in the middle region compared to parotoid gland extraction. The protein bands of Rm value of 0.84 was absent in the parotoid gland secretion, whereas a protein band with Rm value 0.85 was absent in the parotoid gland extraction nearer to molecular weight 14.5 KDa in the fast moving zone (Zone. C).

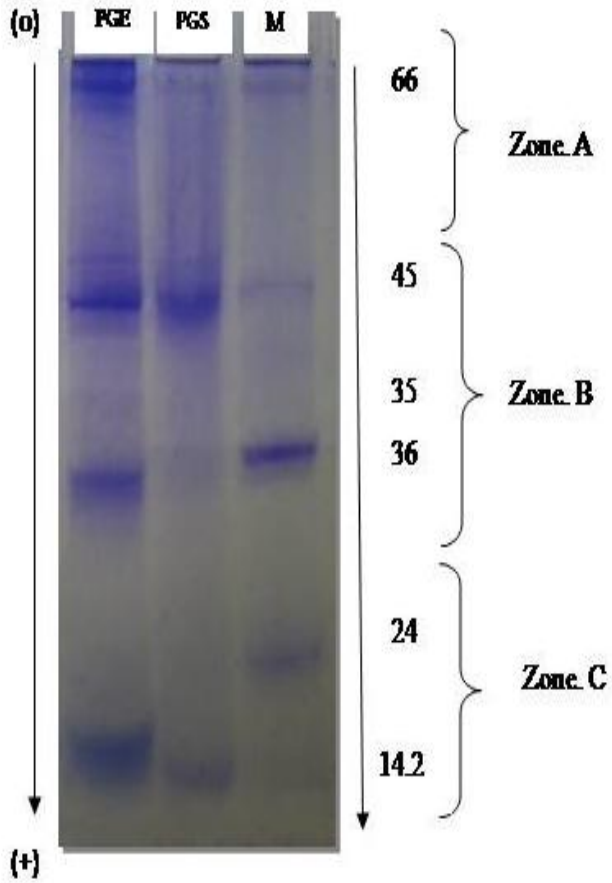

Figure 1. SDS-PAGE Electrophoretic Patterns of Proteins of *Bufo melanostictus* Parotoid Gland Extract and Secretion stained with Coommassie brilliant blue

Left lane indicates (M) mol. weight strands (66-14.2 KD.) 'A', 'B', 'C' zones.
PGE = Paratoid gland Extract.
PGS = Paratoid gland Secretion.
M = Molecular weight standards (14 to 66 KD).
Zone A = mol. wt. 66 KD.
Zone B = mol.wt.45 KD, 36 and 35 KD.
Zone C = mol.wt. 24KD, 14.2K.D.
O = origin.
+ = Anode.
↓ = Direction of run.

Marker	Parotoid Gland Extraction	Parotoid Gland Secretion
0.07	0.07	0.07
-	0.11	-
-	0.12	-
-	0.21	-
-	0.25	-
0.28	0.28	0.28
-	0.31	-
0.50	0.50	-
0.52	0.52	-
0.64	-	-
-	0.84	-
0.85	-	0.85

Table 1. Rm values of Parotoid gland secretion and extract of *Bufo melanostictus* (Schneider).

The patterns of protein bands observed in the parotoid gland extract and its secretion of the toad on SDS-gel indicated a distinct of four protein bands with several additional bands with poor resolution, exhibiting minor variations in the slow moving zone **whereas** a distinct of two protein bands were observed in the parotoid gland secretion. Therefore, the protein patterns observed in the parotoid gland extract and its secretion are more or less similar with minor variations.

The presence of protein bands with identical mobility in the secretions and gland extracts, indicate the similarity of proteins secreted probably by granular cells of epidermis [11]. Various authors have reported that the alkaloids and steroids as toxic and anti feeding agents, acting as a major chemical defense strategy against predators [12, 13]. Such molecules act on the cardiovascular system, raising the blood pressure and/or increasing the contraction force of the heart [14, 15]. The secretary proteins exist as coiled filaments within epidermal granular cells [16]. 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 as used here. For instance, the magainins found in skin secretions of *Xenopus* are typically of 21-26 amino acid residues in length [17].

Conclusion

In view of the above results it can be concluded that the exudates of toad parotoid gland may also contain several of these granular cells and when their electrophoretic patterns were observed on SDS- gel revealed the presence of protein bands with identical mobility both in secretion and gland extract indicating homology of cell lines and its secretion in *Bufo melanostictus*.

The present investigation reports that the analysis of protein patterns of *Bufo melanostictus* on SDS-PAGE, in spite 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.*

Acknowledgement

The authors are thankful to the authority of University Grants Commission (UGC), New Delhi for financial assistance under Major Research Project (MRP) F.No.39-596/2012(SR) and also thankful to the Head, Department of Zoology, Kakatiya University, Warangal, for providing laboratory facilities.

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