

Biochemical Composition of Epidermal Secretions and Poisonous Spine of Two Freshwater Catfishes

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Abstract

Biochemical composition of epidermal secretions and poisonous spine extracts of two freshwater catfishes, *Heteropneustes fossilis* and *Clarias batrachus* were estimated. Protein patterns of the two tissue extracts were compared using SDS polyacrylamide gel electrophoresis. The protein content of epidermal secretions is higher than those of poisonous spine; In *H. fossilis* the difference is about 1.8 folds, but in *C. batrachus*, it is about 8 folds. Lipids of epidermal secretions in *H. fossilis* are about 2.8 folds higher than those of the poisonous spine. Trichloroacetic acid (TCA) soluble peptides, ninhydrin positive substances show significant variation between the two tissues of *C. batrachus* but not in *H. fossilis*. The electrophoretic patterns exhibit more number of protein bands in the extracts of poisonous spine than those of epidermal secretions.

Introduction

The surface mucus produced by the epidermis of fish is known to reduce the turbulence generated during swimming (Rosen and Cornford 1971) and to protect the fish from pathogenic infections (Trust 1986). Two kinds of cell lines are known to contribute to the mucus formation by skin: the goblet cells, which contribute primarily to the production of glycoprotein rich slimy mucus and clavate cells, which contribute to the production of proteinaceous material (Quay 1972, Cameron and Endean 1973). The latter are known to be rich in the epidermis of fishes with reduced squamation and are believed to play an important role in producing the toxic components, *ichthyocrinotoxins* (Halstead 1970). These cells get concentrated at the base of the pectoral spine (Poison glands) in catfishes and aid the fish in defense mechanism (Cameron and Endean 1973). *Heteropneustes fossilis* and *Clarias batrachus* are the two common freshwater catfishes found in India. The

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crude extracts of the pectoral spine of these fishes exhibited several pharmacodynamic activities in experimental animals (Bhimachar 1944; and Datta et al. 1982). The biochemical composition of the mucus or that of the poisonous spine of these two fishes has not been worked out. In an earlier study (Venkaiah 1999), it was demonstrated that the extracts of the two tissues from these fishes induced cardiotoxic activity in isolated hearts of frogs and the extracts from *H. fossilis* had greater influence than those of *C. batrachus*. In view of the importance of extracts of epidermal secretions and poisonous spine in pharmacology, a study was made on the biochemical composition and protein patterns of the tissues of these two fishes.

Materials and Methods

The fishes (20 to 30 cm in length, weighing about 50 to 100 gms.) were collected from the local freshwater tanks (ponds) located within the radius of 15 km from the Kakatiya University Campus. They were immobilized on ice and the epidermal secretions were collected by scraping the surface using a sterile blade and placed in ice jacketed glass petridishes. The pectoral spine along with the basal tissue constituting the poisonous spine was isolated from the fish and placed on ice jacketed containers. The tissues were weighed to the nearest milligram and were processed for further analysis. They were homogenized (10%) in 10% Trichloroacetic acid (TCA) to sediment the protein. The protein sediment was dissolved in 1N NaOH and the protein content was determined through the Lowry's reagent as described by Schacterle and Pollack (1973). The TCA supernatant was used to estimate TCA soluble peptides (Lowry's reagent), Ninhydrin positive substances (Lee and Takahashi 1966) and carbohydrates (Anthrone method, Carroll et al. 1956). Lipid content of the tissues was determined through the chloroform: methanol (2:1) extraction method of Folch et al. (1957). The particulate matter (dry matter) of the tissues were determined by heating the tissues at 110°C from 48 to 72 hours until a constant weight of the dry material was obtained. Water content of the tissue was calculated as the difference between the weight of fresh tissues and that of dried tissues. Students "t" test was used to compare the results between the two tissues of each species.

For the electrophoretic pattern of proteins, two methods viz., Laemmli's (1970) method of SDS-PAGE and Anderson et al. (1983) method (Urea gels) were used. Thin layers (1.5 mm thick) polyacrylamide slab gels (14 x 14 cm) (12% T and 3.6% C) were prepared by using the glass plates. The protein for electrophoretic studies was extracted by homogenizing the tissues (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 2,000 rpm for 20 minutes in a clinical centrifuge at room temperature ($30 \pm 2^\circ\text{C}$) and the supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, 2-mercaptoethanol and bromophenol blue as the tracking dye; 0.1 ml (5 mg) of the tissue extract was loaded on to the separating gel directly. The electrode buffer, 0.025 M Tris and 0.192 M glycine, was used for Laemmli's

method, whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with concentrated HCl as upper chamber buffer and IM Tris, 0.2% SDS adjusted to pH 7.8 with concentrated H_2SO_4 was used for the Anderson et al. method. A constant current of 20 mA for the first 15 minutes followed by 40mA 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 12 cm from the origin. A solvent containing 0.25% Coomassie brilliant blue in methanol: water: acetic acid (5:5:1) was used for staining the proteins separated on gel by Laemmli's method. Silver nitrate (Switzer et al. 1979) was used for staining proteins separated by the method of Anderson et al. (1983). The molecular weight standards used in comparing the variations noticed in the urea gel were low molecular weight protein standards (14 to 66 KD) from the Sigma Chemical Company (USA).

Results

Values obtained from the quantitative estimates of the biochemical composition of the two fishes are presented in Tables 1 and 2. Results show that the epidermal secretions of *Heteropneustes fossilis* and *Clarias batrachus* are rich in lipids and proteins while carbohydrates are very low. There is a significant difference in these components between the epidermal secretions

Table 1. Biochemical constituents of epidermal secretions and poisonous spine of *Heteropneustes fossilis* (Values are expressed as mean $\pm$ SE mg/100 mg of fresh weight of observations of six replicates of each species; lipids* are expressed as mg/100 mg of dry weight).

S. No.	Item	Epidermal secretion	Poisonous spine extract
1.	TCA precipitated proteins	0.723 $\pm$ 0.063	0.392 $\pm$ 0.043*
2.	TCA soluble peptides	0.113 $\pm$ 0.027	0.149 $\pm$ 0.027
3.	Ninhydrin positive substances	0.465 $\pm$ 0.444	0.578 $\pm$ 0.143
4.	Carbohydrates	0.040 $\pm$ 0.086	0.085 $\pm$ 0.029
5.	Lipids *	54.507 $\pm$ 3.206	19.094 $\pm$ 1.564*
6.	Water content	92.062 $\pm$ 1.394	47.796 $\pm$ 6.538*
7.	Dry weight	7.408 $\pm$ 0.830	52.471 $\pm$ 6.499*

Asterisk (*) denote significant difference at $p < 0.005$

Table 2. Biochemical constituents of epidermal secretions and poisonous spine of *Clarias batrachus* (Values are expressed as mean $\pm$ SE mg/100 mg of fresh weight of six observations of six replicates of each species; lipids* are expressed as mg/100 mg of dry weight).

S. No.	Item	Epidermal secretion	Poisonous spine extract
1.	TCA precipitated proteins	1.375 $\pm$ 0.16	0.166 $\pm$ 0.027*
2.	TCA soluble peptides	0.305 $\pm$ 0.049	0.149 $\pm$ 0.049*
3.	Ninhydrin positive substances	0.488 $\pm$ 0.020	0.63 $\pm$ 0.076*
4.	Carbohydrates	0.065 $\pm$ 0.005	0.066 $\pm$ 0.022
5.	Lipids *	8.264 $\pm$ 0.277	9.521 $\pm$ 0.022*
6.	Water content	82.079 $\pm$ 0.780	46.872 $\pm$ 2.948*
7.	Dry weight	18.186 $\pm$ 0.687	53.127 $\pm$ 2.948*

Asterisk (*) denote significant difference at $p < 0.005$

and poisonous spine of the two fishes. The TCA soluble peptides, ninhydrin positive substances do not show any significant variations between the two tissues in *H. fossilis*, although in *Clarias*, the variation is significant. Particulate matter (dry weight) is high in the poisonous spine. This is due to the contents of spine present in the extract. There is a significant variation between the particulate matter of epidermal secretions of *H. fossilis* ($\approx 7\%$) and *Clarias batrachus* ($\approx 18\%$). The lipid content of the epidermal secretions of *H. fossilis* is very high (54% of dry weight) when compared to that of the poisonous spine ($< 20\%$ of dry weight). The lipid composition of the two tissues also exhibits significant variations between the two species. *Clarias batrachus* has less lipid content ($< 10\%$ of the dry weight) in both the tissues. Preliminary studies on lipid composition using TLC technique revealed that majority of the lipids stain positively with phosphate and ninhydrin stains (Venkaiah and Lakshmipathi, Unpublished data).

Electrophoretic separation of proteins following Laemmli's methods stained with Coomassie brilliant blue (Fig. 1) demonstrated the presence of about 15 - 20 protein bands in the extract of poisonous spine of the two fishes with three to five prominent bands. In epidermal secretions, the pattern yielded only few bands (3 - 5) stained very lightly and not clearly visible in the photographs. Except for minor differences in the mobility of some bands, the pattern is similar in both the fishes. The pattern observed with SDS-Urea gels stained with Silver nitrate (Fig. 2), however, indicated distinct differences in the protein bands of the poisonous spine. Comparison of the protein bands of variable regions with the standard marker proteins revealed that variation is higher in the region of slow moving zones, "A", (Mol. Wt. > 66 k.D) and those with fast moving zones, "C" (Mol. Wt. < 24 k.D). The pattern obtained in the middle region, "B" (Mol. Wt. 66-24 k.D), is more or less similar in the two species. Epidermal gel, when loaded at higher concentration (10 mg of the extract), yielded distinct bands in this region. The number of bands noticed were very few but they were similar to the bands observed in the poisonous spine in the two species.

Discussion

Reviewing the chemical composition of the venom and epidermal secretions of *Arius thalassinus* (= *Arius bilineatus*), Al-Hassan et al. (1987) reported that the material is rich in proteins and lipids, but very low in carbohydrates. Fish mucus was shown to be a rich source of lipids, which is 20 times higher than that of human sebum. Contribution of the lipids in mucus from a single catfish was shown to be more or less similar to those of the 15 specimens (individuals) of mullets (Lewis 1970). Lipids were shown to be the principal constituents of the skin of the catfishes *Clarias batrachus* and *Heteropneustes fossilis* by histochemical methods (Benerjee and Mittal 1975; and Mittal et al. 1976). The lipid-protein ratio in the mucus secretion of *Clarias batrachus* was shown to be 0.06 (Mittal and Nigam 1986). Most of these lipids were phospholipids (Lewis 1970; and Mittal and Nigam 1986).

More than thirty proteins in venom and fifty proteins in epidermal secretions were reported in marine catfish on the SDS gels by Al-Hassan et al. (1987). Proteins in the epidermal secretions were shown to form an insoluble matrix and required high concentration of ionic detergents for their solubilization. Comparisons of the protein and lipid composition of the epidermal secretions of three marine catfishes (Genus *Arius*) from the Arabian Gulf (Ali et al. 1989), revealed that the catfishes exhibit a general similarity in composition of proteins, although some minor differences were shown to exist in the size and number of proteins. Lipids were shown to be variable with season, diet and the species comparisons were largely offset by these differences. The high protein and lipid contents and low carbohydrate contents of the epidermal secretions and venomous spine extract in the present investigation are in general agreement with the reports on these catfishes. Electrophoretic patterns of the proteins, however, reveal that very low amounts of protein in epidermal secretions are soluble in the extracting media used for

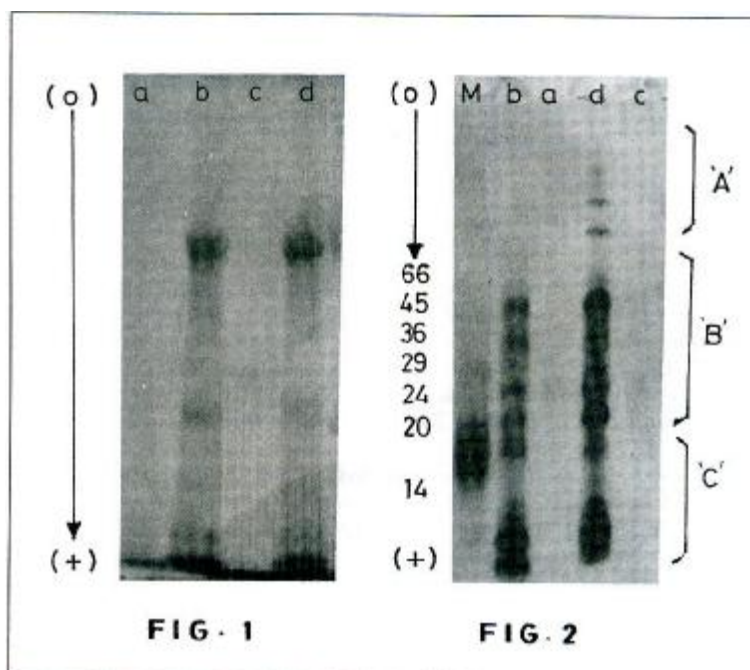


Fig. 1. Electrophoretic patterns of proteins on SDS-PAGE (Laemmli method) stained with Coomassie brilliant blue.

Fig. 2. Electrophoretic patterns of proteins on urea gels (Anderson *et al.* 1983) stained with silver nitrate; left lane (M) mol. weight standards (66-14 k.D.) 'A', 'B', 'C'. Regions of similarity and variability of protein patterns between the two species.

- a = Epidermal secretions of *H. fossilis*.
- b = Poisonous spine extract of *H. fossilis*.
- c = Epidermal secretion of *Clarias batrachus*.
- d = Poisonous spine extract of *Clarias batrachus*.
- M = Molecular weight standards (14 to 66 k.D.).
- O = Origin
- + = Anode
- ↓ = Direction of run.

the isolation of proteins. We could observe a good amount of sedimentation in the centrifuge tubes of these extracts. It is possible that much of the protein is lost in insoluble form. Poisonous spine extract, on the other hand, contains many of the soluble proteins. Describing the histology of the poisonous spine of *H. fossilis*, Bhimachar (1944) indicated that there is no venomous sac in this fish. Cameron and Endean (1973) suggested that the concentration of proteinaceous material secreting cells around the pectoral spine led to the evolution of venomous gland in catfish. Rupture of this epithelium during envenomation is the source of the venom injected into the victim's body. Similarity of certain protein bands observed in epidermal secretion and poisonous spine in electrophoresis in the present investigation and those reported for *Arius bilineatus* (Al-Hassan et al. 1987) can be attributed to the similar cell line contributing to these protein products in the two tissues.

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