

COMPARATIVE ELECTROPHORETIC STUDIES OF LENS PROTEIN ISOLATED FROM PUNTIUS TICTO (HAMILTON 1822) AND RASBORA DANICONUS (HAMILTON 1822)

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ABSTRACT

A comparative electrophoretic study of the eyelens protein of two freshwater fishes viz. *Puntius ticto* and *Rasbora daniconus* was carried out. Electrophoretic analyses revealed substantial differences in the polypeptide distribution in both the fishes. SDS-PAGE of water soluble fractions of lens protein showed several bands ranging from 70-13.5 kDa and 74-13.5 kDa in *P. ticto* and *R. daniconus* respectively. The insoluble protein fraction was further resolved into the urea soluble lens protein (USLP) and plasma membrane protein fraction (PMP), SDS-PAGE analysis of the USLP fraction showed the presence of 6 bands ranging from 120-13.5 kDa in *P. ticto* and 114-13.5 kDa in *R. daniconus*. The PMP fraction of *P. ticto* resolved into 6 subunits ranging from 74-13.5 kDa. In contrast only two bands corresponding to 29 and 13.5 kDa was observed for the PMP fraction of *R. daniconus*. The present study demonstrates a complete variation in the protein pattern of the soluble and insoluble protein fraction in these two fishes. The variation in eye lens protein which are considered to be a conserved protein suggest that the *R. daniconus* had undergone gene duplication in the course of evolution and the two fishes may have evolved differently.

INTRODUCTION

The eye lens is a unique part of both the human and the animal body. It is different in many aspects from any other soft tissue. The main feature of the normal lens that is crucial for sight is its transparency. It appears that natural selection guided eye lens features towards a structure and components capable of preserving transparency of the lens for many years, giving evidence of the possible presence of reactive species and exposure to visible and UV radiation (Augusteyn and Stevens, 1998). One of the lens components are lens itself and have essentially soluble component. The eye lens is a unique, avascular, non-innervated structure that functions to deviate light onto the retina and make small adjustments in focus by accommodation (Augusteyn and Stevens, 1998). In order to refract light effectively, the lens must remain transparent. Lenses contain protein called crystallin that accounts for around 90% of the soluble proteins in the lens and play a crucial role in maintaining lens transparency (Bloemendal, 1977; Delaye and Tardieu, 1983; Wistow and Piatigorsky, 1988; Ponce et al., 2006). In the vertebrate lens, three main classes of crystallins are recognized viz. α , β and γ (Harding and Dilley, 1976).

Lens proteins from a range of species have been studied to provide information on their evolutionary history (Wistow and Piatigorsky, 1988; Chiou et al., 1986; De Jong and Hendriks, 1986). Though soluble lens proteins have been investigated extensively in a range of mammals, information on lens protein derived from fishes and amphibian lenses is limited (Cobb

et al., 1969; Mehta and Lerman, 1971; De Jong et al., 1975; Zigler and Sidbury, 1976; De Jong et al., 1977; De Jong and Bloemendal, 1981; Bindels et al., 1983; Chiou et al., 1986; Chiou et al., 1988; Keenan et al., 2012). γ -crystallins are known to be the main lens proteins in lower vertebrates such as amphibians (Brahma and Van Doorenmaalen, 1969; Keenan et al., 2012). The common crystallins exist in all vertebrate lenses, however, additional taxon-specific crystallins are present in lenses of particular species (Piatigorsky, 1989; De Jong et al., 1989).

It was in 1980's that insoluble components received attention and ever since the role of soluble, insoluble, with urea and plasma membrane protein have been studied in depth. However, with increasing information available the study is leading into confusion. Thus, in this context the present study was carried out to compare the lens protein from two freshwater fishes, namely *Puntius ticto* and *Rasbora daniconus*

MATERIALS AND METHODS

The fishes were procured from Nathsagar dam, Paithan, near Aurangabad. The fishes were acclimated in the laboratory conditions for two weeks before the commencement of the experiments. The fishes were fed *ad libitum*. The fishes used for the present study were, *Puntius ticto* and *Rasbora daniconus*.

Preparation of lens protein

The lens from these fishes were dissected in chilled 0.05M Tris HCl buffer pH 7.5 and homogenized in glass homogenizer.

The procedures adapted were as described by Kibbelaar and Bloemendal (1975). About 10 extracts were obtained for each lens. Each extract was centrifuged at $11000 \times g$ for 20 minutes at 4°C to separate water soluble protein from water and urea insoluble fractions. Protein content of the soluble and insoluble fractions were determined by UV Spectrophotometer at 280nm, using BSA as standard. All the measurement was carried out in triplicates.

Preparation of lens cortical proteins

Lens cortices are homogenized in 0.05 M Tris-HCl buffer at pH 7.5, centrifuged at $15,000 \times g$ and the pellet was washed thoroughly so that the last wash does not contain material absorbing at 280nm more than 0.05 mg/mL. The final pellet was suspended in 0.05 M Tris-buffer containing 6M urea, 0.05M NaCl and 0.001 M EDTA and adjusted to pH 8.6. The suspension was stirred for 2 to 3h. All work were carried out at 4°C . Thereafter the solution was centrifuged $20000 \times g$ for 150 minutes. The supernatant contains the USL fraction.

Preparation of lens membranes

The urea-insoluble fraction of fish lens contains a considerable amount of plasma membranes, containing typical intrinsic proteins. For the preparation of the lens plasma membrane fraction, the method as described by Kibbelaar and Bloemendal (1975) was followed. This method is virtually confined to water and urea extraction and is based merely upon the (in) solubility properties of the membranes. The density and solubility properties of the membranous material were not explored in the present study.

SDS- electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using Microkin unit, Techno source (Mumbai). Crystallin protein (10–20 μg per sample) was loaded on a discontinuous SDS-polyacrylamide gel (4% stacking gel, 7.5% resolving gel) and electrophoresed for 2h at a constant voltage of 150 V. Gels were stained with 0.1% Coomassie R-250 in 10% acetic acid, 40% methanol and destained with 10% acetic acid, 40% methanol. Samples from each preparations, described above were subjected to SDS-PAGE. The standard Molecular markers (medium range 97–14.3kDa) were used as supplied by Merck, India.

RESULTS

In the present study the fish lens protein were divided into soluble fraction containing crystallin, an insoluble fraction containing crystallin and cyto-skeletal proteins, The insoluble fraction was further analyzed for protein by using urea soluble fraction and plasma membrane fraction. SDS-PAGE pattern of lens proteins of fishes is shown in Fig. 1. The crystallin fraction in *P. ticto* showed four bands in SDS-PAGE viz. 70, 38 29 and 13.5kDa the urea insoluble fraction in *P. ticto* contained protein bands of 120, 70, 36kDa, 28, 24 and 13.5 kDa. and *R. daniconus* showed protein bands of 114kDa, 74kDa, 34.6kDa, 27kDa, 24kDa and 13.5kDa. The lens plasma membrane showed six subunits i.e. 74 34a, 26, 22 and 13.5kDa in *P. ticto* and *R. daniconus* showed two bands of 29 and 13.5 kDa protein bands.

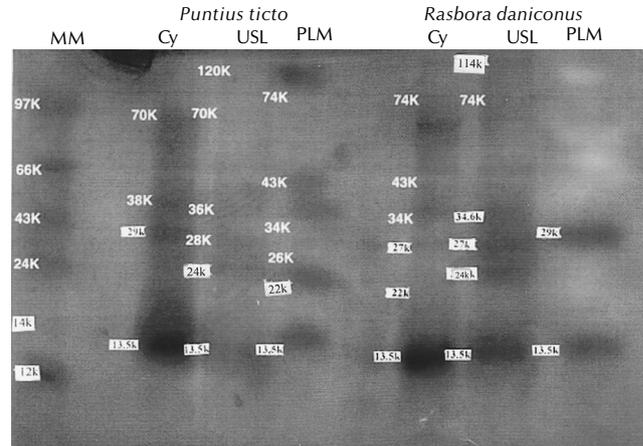


Figure 1: SDS-PAGE of eye lens protein (all fractions) of two fish's *P. ticto* and *R. daniconus*. Lane 1. Standard Molecular weight, Abbreviations. Cy (Crystallin); USL (Urea soluble lens protein; PLM (Plasma membrane protein)

DISCUSSION

We examined the fish lens crystallin fraction, i. e. water soluble and water insoluble fraction in the present study. As with other animals, the soluble proteins from each of these lenses are composed of the classical groups of α , β and γ -crystallins found in human and other mammalian lenses (Harding and Dilley, 1976; Bloemendal *et al.*, 2004). The taxon specific crystallin of single polypeptide of molecular weight 38kDa is related to or identical to metabolic enzyme class mostly, the oxido-reductase and are often called as enzyme crystallin (Piatigorsky and Wistow, 1991). Such a crystallin has been reported in amphibians by various workers (Keenan *et al.*, 2012; Tomarev *et al.*, 1984; Fujii *et al.*, 2001; Chiou, *et al.*, 1986; Lu, *et al.*, 1995) and is known to belong to reductase super family. β - Crystallin is a large and diverse group of lens crystallin. Comparative studies of Zinger and Sidbury, (1976) have shown a common occurrence of the number of polypeptides in the β -crystallin of various mammalian and sub mammalians. Among these the most common polypeptide are two with apparent molecular weight of 26 and 28 kDa. The 26 kDa polypeptide appear to be the same as the polypeptide designated βBp by Herbrink and Bloemendal, 1974. Later investigators reported the molecular weight of 27kDa as a major molecular weight common to both the βH and βL crystallin. A similar protein was reported in Guinea pig by Huang *et al.* (1987). In the present study the crystallin proteins of *P. ticto* showed three bands 70, 38 and 29kDa and *R. daniconus* showed six bands of 74, 43, 34, 27 and 22kDa respectively. We observed a relative abundance of two polypeptides, 27 kDa and 22kDa in *R. daniconus* and in *P. ticto* a 29kDa polypeptide. It appears that the accumulation tendency of 22kDa polypeptide may be either with age or changes in Na^+ and K^+ ratio's. Though in the present study age was not considered, therefore, we cannot draw conclusion, but rather speculate. Since the 27 and 29kDa polypeptide occur together as subunits of β_3 subunits, their changing ratios suggest that they can assemble in different proportion to make up the β crystallin molecules in a manner analogues to a non enzymic structure. Jiang *et al.* (1989) reported 28kDa as a non glycosylated band in frog and tadpole lenses

and it corresponds to β -crystallin. Jiang *et al.* (1989) speculated that this 28 kDa may correspond to bovine lens B β crystallin, which is similar to main β -crystallin in bovine lenses. In the present study we believe that the 29 and 27 kDa may represent the β -crystallin as suggested by Jiang *et al.* (1989). The *P. ticto* and *R. daniconus* showed different bands, suggesting that crystallin proteins may have evolved by gene duplication during evolution. Thus, it appears from the studies that the two fishes belonging to family, Cyprinidae show heterogeneity.

The insoluble protein from eye lens is categorized into urea soluble lens protein (USLP) and Plasma membrane protein (PLM). USLP often contains crystallin and cytoskeletal proteins. We found some of the crystallin protein in USLP fractions, such as 70, 28/29 in *P. ticto* and 74, 34 27kDa in *R. daniconus*. Thus we observed crystallin proteins bands of 38 and 29 kDa in *P. ticto* and in *R. daniconus* 43 and 22 kDa. Similar observations were reported in water soluble and USLP fraction by Bloemendal, 1982.

The cytoskeletal protein observed in *P. ticto* and *R. daniconus* were 120, 36, 28, 24, 13.5 and 114 and 24kDa respectively. The presence of the 120 and 114 kDa subunits for the cytoskeleton proteins has not been reported so far. A 115 kDa the role of 120 and 114kDa obtained from urea soluble cytoskeletal protein in the present study is not known. Similarly, the other proteins particularly primary role of protein 36, 28, 24kDa in *P. ticto* and 24 kDa in *R. daniconus* needs further investigations.

SDS-PAGE of urea extracted lens plasma membrane revealed the relative enrichment of membrane insoluble protein (MIP) and the urea insoluble membrane associated with protein of molecular weight 34 and 26kDa. A 38 KDa protein was identified in guinea pig (Huang *et al.*, 1987) and camel (Rao and Zigier, 1990). In Guinea pig 35 KDa is a ξ crystalline and is a NADH dependent quione oxido-reductase (Rao and Zigier 1990; Rao *et al.*, 1992). The exact role of this protein in *R. daniconus* is not certain. However, this molecule is known to maintain the lenticular environment in a reduced state. It is also not known if, it represents integral or strongly bound peripheral membrane proteins (Kistler *et al.*, 1985). The 22 kDa proteins observed may have arose due to ageing or artifact of 26 kDa protein as reported by Roy *et al.* (1979).

The plasma membrane protein in *R. daniconus* showed a single protein subunit of 29 kDa. The function or the significance of 29 kDa proteins observed in the present study is not known. The membrane protein 34 kDa is also an intriguing transmembrane constituent. This protein is found in both, in plasma membrane isolated from epithelial cells and lens fibers (Vermorken *et al.* (1979). Whereas, Membrane protein 26 (MP26) appears to be a typical fibre cell membrane component, presumably the major protein component of the larger junctional structures. It appears that probably this protein is synthesized exclusively in lens fibre cells. The function and biochemical role of 74 kDa protein in *P. ticto*, is presently unknown. Lens proteins are inserted in membranous structure and is associated with heterologous membrane (Vermorken *et al.* 1979). The 26 kDa the main lens membrane intrinsic polypeptide is believed to be the major component of fibre gap junction. In all the preparations of the lens fraction studied, an unidentified polypeptide of molecular weight 13.5 kDa

was observed. The function of this protein is unknown. A similar observation was reported by Bloemendal (1982).

Thus, we conclude that, though both the fishes belong to Cyprinidae family, the protein derived from eye lens showed an unusual variation. This suggests that, the two fishes might have evolved independently. The fish crystallin soluble fraction was earlier used to study the evolutionary pattern. The role of chaperones and enzymic activity of crystalline protein warrant further investigations in lower vertebrates where studies are scarce. Other proteins from lens such as seen in urea fraction, plasma membrane protein may pave an interesting model to understand lens differentiation in aquatic vertebrates especially fish.

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