Asian J. Exp. Sci., Vol. 19, No. 1, 2005, 59-64

Qualitative Polyacrylamide Gel-Electrophoretic Analysis of Retinal Proteins in Vertebrates

S.C. Maiti,*+# C.R. Maiti and S.K. Ghosal*****

* Zoology Department, Sitananda College, Nandigram, East Midnapore, West Bengal

** Health and Medical Education, Govt. of West Bengal, Kolkata 700091

*** Zoology Department Burdwan University, Burdwan, West Bengal 713104

Abstract : Polyacrylamide gel electrphoresis of vertebrate retinal tissue was done. The protein profiles of *Labeo rohita, Bufo melanostictus* and *Calotes versicolor* suggest a tendency for an increase of differently sized fragments sorted thusfar. The basic banding pattern, however, is retained upon the garden lizard. The White Leghorn retina, in sharp contrast to the goat one, displays not only the widening of certain common bands, but also the emergence of atleast three additional bands, presumably accounting for the complexity and acuity of highly evolved avian vision. Such bifurcation of avian and mammalian banding patterns from a typical reptilian profile is of paramount phylogenetic significance.

Key words : Protein profiles, retinal tissue, *Labeo rohita, Bufo melanostictus, Calotes versicolor,* polyacrylamide gel electrophoresis

Introduction :

Photochemical receptors occur in several plants and animals. The animal photoreceptors display absorption maxima within visible spectrum (400-760 nm) of solar radiation. Human rhodopsin (visual purple), a retinal rod pigment, for example, has a 'maximum' of 497nm. Avian **iodopsin** (a cone pigment) and several pigments in human cone have differing maxima accounting for colour vision (Pirenne 1967; Rhushton and Henry, 1968). The sensory response, essentially a light induced "bleaching", involves interconversion of pigment forms with concurrent electric flux propagated by chemical transmission from photoreceptors to prime neurons. This interesting phenomenon prompted us to think with retina of different vertebrates, having diverse pigments delineated Rhuston's (1962) monograph. With the sophistication of expertise and methodologies eye proteins are being analysed for their origin and function. An SDS – PAGE of bovine cornea specific protein (CSP) yields one band (54,000 daltons),

Corresponding Author

while isoelectric as well as chromatofocusing show four bands each (Yumiko, 1990). Unfortunately, the four components have not been biochemically differentiated and the authors negated CSP's glycoprotein nature.

As qualitative Page analysis of vertebrate retinal protiens has not hitherto been studied, the present investigation was aimed to visualise protein profiles of vertebrate's and was planned for having a phylogenetic insight of the kinship of these protein entities from fish to mammals.

Materials and Methods :

Ten eyes from each of the respective vertebrate types, viz, those collected from teleost fish *Labeo rohita* (a fresh water fish), *Bufo melanostictus* (an amphibian), garden lizard *Calotes versicolor* (Reptile), white leghorn (a bird) and a goat (mammal) represent different vertebrate classes used in the present study. All eyes were freshly collected, excised, and the retinal tissues were dissected out, individually weighed separately, homogenised and 2% solution in distilled water was made. Solutions were centrifugel (3000 RPM for 10 min.) and supernatants used for qualitative and quantitative tests for proteins. Employing a polyacrylamide gel electrophoresis (PAGE) apparatus (Quick - fit - Inc.) with LKB 2197 electrofocusing constant power supply, modified method of Davis (1964) was applied. For quantitative test. Folin reagent method was employed. The spectrophotometer measured the O.D. against the blank and the data were plotted graphically with consequent estimation of protein per ml.

From the other half of the samples, gel electrophoresis was made by dilution which possessed nearly 100 - 150 μg of protein 0.2 ml of each solution. Polymerization of small pore gel solution (30%) and large pore solution (7.5%) was performed. Sample proteins were initally poured over the large pore gel, each gel tube being covered apically with a drop of 50% sucrose, and Bromophenol blue as indicator for movement of protein molecules through the disc which was mixed with the tris-glycine buffer in the upper chamber (connected with cathode). The lower chamber (connected with anode) too was filled with tris-glycine buffer.

The current was distributed to each tube initially 1 m.A. and crossing large pore gel, increased to 3 m.A. per tube at 5° C. The process was complete when indicator dye disc reached the bottom of each tube. Gels were removed from the tube and stained in 1% coomassie brilliant blue to colorise the protein bands in the gel. The detailed banding pattern with width and intensity was presented diagramatically and photographically. In both the cases, standard human serum protein bands were compared.

Observation :

The gel profile from fish to reptile exhibited a gradual tendency for an increase in retinal protein bands. Here the Rohu fish and amphibia (toad) have 2 and 3 bands, respectively, whereas reptile *(Calotes)* has 6, aves (chick, white leghorn) I bands constrasting mammal (goat) with 4 major protein bands. Moreover, the white leghorn, in sharp contrast to the goat, displays profound widening of certain bands testifying presumably to the complexity and acuity of highly evolved avian vision. (Fig. 1 and 2).

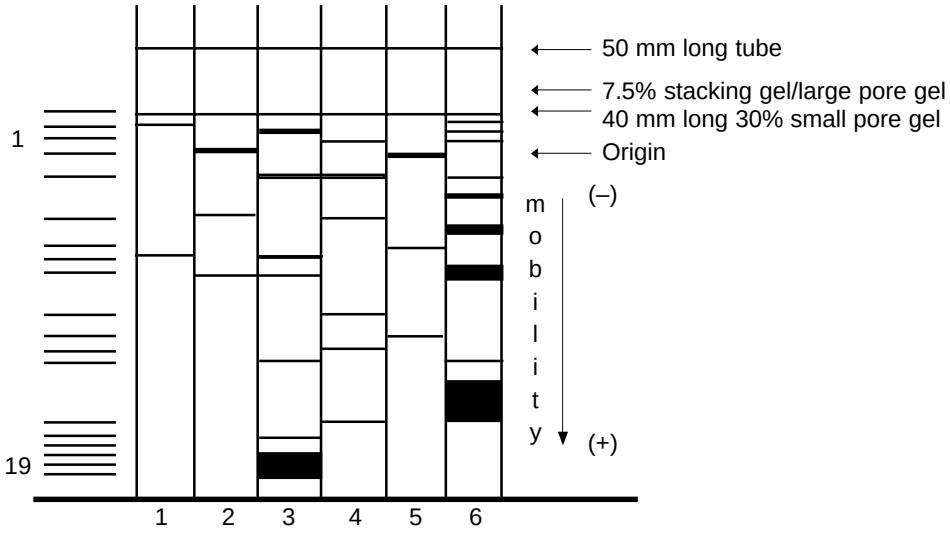

Fig. 1 : Diagrammatic representation of electrophoretic pattern of eye proteins in retina of different vertebrates

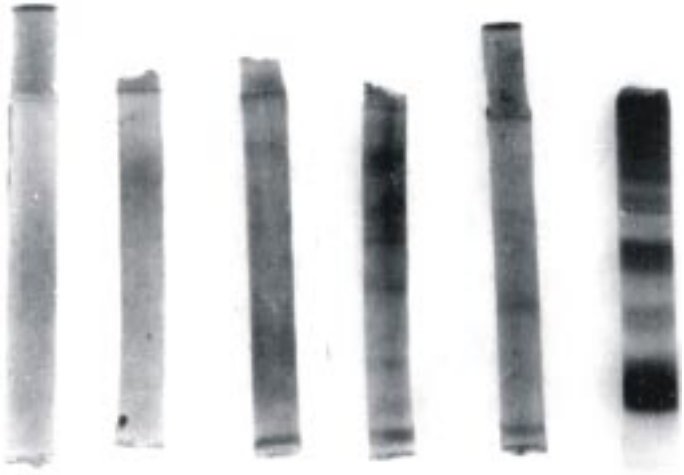

Fig. 2 : Photo representation of electrophoretic protein of eye protein in retina of different vertebrates serially. 1. Rohu, 2. Toad, 3. Calotes, 4. bird, 5. Goat, 6. Standard human serum.

Discussion :

The enzymatic activities are as much closely associated with vision physiology as the retinal complexity. Recoverin, a recently discovered 26 KDa calcium-binding protein activates guanylate cyclase in retinal photoreceptors when the intracellular free calcium concentration drops upon photoexcitation. Its role though well-known for phototransduction in light sensitive pineal orgen of poikilothermic vertebrates and birds. (except for the chickens), is debatable in mammalian pineal organ, which is not photosensitive (Korf *et al*., 1992).

It seems that it may be a component of band 2 of the present study, while, band 1 may represent a higher molecular weight protein kinase C (occurring as isoenzme α, β, γ) whose existence has been detected by PAGE and Western blotting (Osbrne *et al*., 1992) found from fish to mammal. The presence of recoverin in both retina and pineal organ of Xenopus laevis larva, one day old chicken, adult pigeon, albino rat, sheep and man, and its fairly high molecular weight may suggest its highly conserved nature in the course of phylogeny.

Retinal protein has several following proteins, such as (i) Serine protease, (ii) Tubulin, (iii) the cilium, (iv) D -amino -oxidase, (v) Acetylcholinesterase and butyrylcholinesterase, and (iv) retinyl ester hydrolase.

(i) A 70 KDa serine protease with an alkaline pH optimum, besides other proteases in carp retina was detected in polyacrylamide gels, paralleling proteases found in the central nervous system (Floyd *et al*., 1992).

(ii) Quantification by gel densitometry of tubulin, a 58 KDa protein doubtlet in bovine retinal rod outer segment (ROS) showed that total amount of tubulin was 5 to 10 fold higher than that attributable to the rod axoneme suggesting additional role for tubulin in photoreceptor cells (Matesic *et al*., 1992).

(iii) The cilium of rod photoreceptors functions through actin-myosin contractile mechanism operating the formation in of new photoreceptor disc membranes (Williams *et al*., 1992)

(iv) D-amino-oxidase in peroxisomes of frog cones and Miiller cells may participate at lipid metabolism in neural retina (St. Jules *et al*., 1992) and oxidation of transmitter -related aminoacids and of other small molecules.

(v) During the embryonic chick retinal development, butyrylcholinesterase activity decreases, while acetylcholinesterase activity increases 13 times that the other (Salceda and Martinez, 1992)

(vi) The bovine ocular tissues contain several retinyl ester hydrolases (Tsinal., 1992).

In rat eye, ocular retinol-protein (RBP) and transthyretin (TTR), also synthesized by RPE (Retinal pihmented epithelium) may function in the intraocular translocation of retinol (Joseph *et al*., 1992).

Parvalbumin a calcium - binding protein, is distributed in the retinae of different vertebrate species (in neuronal cell types within parenthesis) as follow :

Pigeon (amacrine, ganglion and bipolar cells); owl (bipolar cell); teleost (amacrine and ganglion cells); rabbit (amacrine, ganglion and horizontal cells); hamster (amacrine cells); ground squirrel (amacrine and

ganglion cells); cat and baboon (amacrine, ganglion and horizontal cells) (Paolo *et al*., 1993).

The present investigation not only reiterates (a) the harmony of the protein profiles from fish to reptile, and a bifurcation of electrophoretic banding during the phylogeny of birds and mammals from a typical reptilian stock, but also (b) the great evolutionary complexity and visual acuity as selected by natural selection.

References :

Davis Floyd D.N.A. shall F. and Djamgoz M.B.A. (1992) : Protease activities in Carp retina. Neurochem. *Int*. **21(4),** 527 - 533.

Joseph H., Cavallaro T. and Martone R. (1991) : The distribution of retinal - binding protein and its messanger RNA in the eye. *Invest. ophthalmol. visual Sci.* **32(2),** 302 - 309.

Korf H.W., White B.H., Schaad N.C. and Klein D.C. (1992) : Recoverin in pineal organs and retinae of various vertebrate species including man. *Brain Res*. **595(1),** 57 - 66.

Matesic D.F., Philip N.J., Murray J.M. and Liebman P.A. (1992) : tubulin in bovine retinal rod outer segments. *J. Cell sci*. 103(1) : 157-166.

Osborne N.N., Barnett N.L., Morris N.J.. and Huang F.L. (1992) : The occurrance of three iso-enzymes of protein C (α, β and γ) in retinae of different species. *Brain Res*. **570(1/2),** 161 - 166.

Paolo S.P., Keyser K.T., Celio M.R., Karten H.J. and Bloom F.E. (1993) : Distribution of parvabumin immunoreactivity in the vertebrate retina. *Brain Res*. **600(1),** 141 - 150

Pirenne M. (1967) : Vision and the Eye. 2nd ed. (London) : Chapman and Hall.

Rushton W.A.H. and Henry G.H. (1968) : Bleaching and regeneration of cone pigments in man. *Vision Res*. **8,** 617 - 631.

Rushton W.A.H. (1962) : Visual Pigments in Man. (Liverpool University Press).

Salceda R. and Martinez M.T. (1992) : Characterization of acetylcholinesterase and butyrylcholinesterase activities in retinal chick pigment epithelium development. Exp. *Eye Res*. **54(1),** 17 - 22.

St. Jules R., Kennard J., Setlik W. and Holtzman E. (1992) : Frog cones as well as Miiller Cells have peroxisome. Exp. *Eye Res.* **54(1),** 1 - 8.

Tsin A. Douglas T.C. and Malsbury W. (1992) : Bile salts independent retinyl ester hydrolases in the bovine eye. *Brain Res. Bull*. **28(1),** 121 - 126.

Williams D.S. Hallett M.A. and Arikawa K. (1992) : Association of myosin with the connecting cilium of rod photoreceptors. *J. Cell. Sci*. **103(1),** 183 - 190.

Yumiko K. (1990) : Isolation and characterization of bovine cornea - specific protein. Acta Soc. *Ophthalmol Jpn*. **94(12),** 1184 - 1156.