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Comparision of Eye Lens Protein of Two Different Species of Fish

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Abstract

The Vertebrate Eye Lence Protein High Conc Of Crystalline The Dence Lenses Of Fish Are Particularly Abundant In A Class Called Ym-Crystallin Whose Member Are Characterized By An Usually High Methionine Content And Partion Loss Of The Four Tryptophan Residues Conserved In All Y -Crystallins From Mammals Which Are Proposed To Contribute To Protection From Uv-Damage .Estimation Of Protein By Lowry's Method And Then A Comparative Electrophoretic Study Of The Eye Lens Protein Of To Fresh Water Fishes Viz, (P.Ticto And R. Daniconius) Was Carried Out Electrophoresis Analysis Revealed Difference In The Polypeptide Distribution In Both The Fishes . Sds Page Of Lens Protein Showed Several Bands Of Different Range . The Present Demonstrate A Complete Variation In The Protein Pattern Of The Soluble And Insoluble Protein Fraction In These Two Fishes.

Keywords: Fish, Eye Lence Protein, P. Ticto, R. Daniconius

Introduction

Vision Is An Important Source Of Information For Many Animal, There Is Two Refractive Element In The Vertebrate Eye Cornea And The Crystalline Lens . Elongated Fiber Cells Make Up Vertebrate Eye Lenses, And Three Main Classes Of Proteins—A, Γ -Crystallin Essentially—Make Up Approximately 90% Of All Soluble Proteins. Lin And Team, 2013) These Crystallins Can Remain In The Eye Lens For An Extended Period Of Time With Little Turnover. Despite The Fact That The Crystallin Molecule Undergoes A Variety Of Post-Translational Modifications (Hoehenwarter Et Al. Al., 2006; 2004 Bloemendal). The Vertebrate Eye Lens's Most Important Protein, Alpha-Crystallin, Has Been The Subject Of Extensive Research. The Improvement Of Focal Point In Vertebrates Has Gotten The Most Consideration Than Some Other Tissues. Eye Fundamentally Comprises Of Focal Point, And Advancement Focal Point Is One Of The Fascinating Issue Of Improvement Researcher. The Focal Point Contains A Moderated Proteins And Have Been Utilized By Developmental As Well As Sub- Atomic Researcher To Grasp Transformative Cycles. Basically Talking, Eye Is A Significant Organ Of Vision In All Vertebrates. Eye Focal Points Of Vertebrates Are Made Out Of Lengthened Fiber Cells, Of Which Roughly 90% Of The Absolute Solvent Proteins Has A Place With Three Significant Classes Of Proteins For Example A , ß And Γ-Crystallins Basically. (Lin Et Al., 2013) These Crystallins Can Exist In The Eye Focal Point With Little Turnover All Through The Whole Life Expectancy, But With Different Levels Of Post Translational Changes On Glasslike Atom. Alpha-Crystallin, A Significant Protein Part Of The Vertebrate Eye Focal Point Has Been The Subject Of Extraordinary Examinations Concerning Its Construction And Capability. The Prior Examinations Have Discovered That The Focal Point Are Comprised Of Proteins And These Protein Are Profoundly Preserved In Every Vertebrate Phylum. The Focal Point Have Dissolvable And Insoluble Part And This Insoluble Parts Definitely Stand Out Since, 1980s. Since, Than The Job Of Solvent, Insoluble, With Urea And Plasma Film Protein Were Concentrated On Top To Bottom. Nonetheless, With Expanding Data Accessible The Review Is Driving Into Disarray. This Is A Direct Result Of Numerous Areas Where Data Is As Yet Deficient. It Isn't So Just Human Experiences Waterfall It, Yet All Vertebrates Experiences Waterfall. Accordingly, It Is An Omnipresent Issue Which Need Consideration. The Biochemical Changes Accordingly Different Prompts Need Considered. It Was In 1980's That Insoluble Parts Got Consideration And From That Point Onward Than The Job Of Solvent, Insoluble, With Urea And Plasma Film Protein Were Concentrated On Top To Bottom. Not With Standing, With Expanding Data Accessible The Review Is Driving Into Disarray. Accordingly, In This Setting The Current Review Was Completed To Think About The Focal Point

Protein From Two Freshwater Fishes In Particular (P .Ticto And R. Daniconius).

Review of Literature

Eye Is An Important Source Of Information For Many Animals And A Variety Of Different Eye Types Have Evolved (Land And Nilsson, 2002). The Cornea And The Crystalline Lens Are To Important Refractive Element In Eye (Yakir, Et. Al., 2008). In Aquatic Vertebrates The Cornea Is Surrounded By Water On The Outside And The Watery Aqueous Humor On The Inside. Both Media Have Relatively High. Refractive Index And If The Cornea Is Thin; Which Is The Case In Most Species, Then Its Refractive Power Is Negligible (Mandelman And Sivak, 1983; Matthiessen, 1986). The Vertebrate Lens Is Derived Embryologically From An Invaginated Ectodermal Epithelium, The Lens Vesicle And Grows Throughout Life By The Orderly Proliferation And Differentiation Of Epithelial Cells Into Layers Of Extremely Elongated Fiber Cells (Bloemendal, Et. Al., 2004). Cell Organization Is Important For Lens Transparency And Focusing, But Most Of The Refractive Power Of The Lens Is Conferred By High Concentrations Of Proteins, With Any Highly Abundant Protein Being Designated A Crystallin. The Most Widespread And Apparently Ancient Crystallin Found In Vertebrate Lineage Are The A, □ And Γ Crystallin (Christine, Et. Al., 2013). A-Crystallins The Original Function Appears To Be In Protein Homeostasis As They Belong To The Family Of Small Heat Shock (Stress) Proteins That Are Ubiquitous Across All The Domains Of Life. (Haslbeck, Et. Al., 2005; Mchaourab, Et. Al., 2009: Basha, Et. Al., 2012). The □ And Γ Crystallin Are Not Related To A-Crystallins But Are Members Of Another Protein Super Family Of Restricted Phylogenetic And Tissue Distribution. In Vertebrates, B And Γ Crystallins Are Highly Expressed In The Lens, With Low Levels Found In Some Other Eye Tissues, Particularly In Different Retinal Cell Types (Sinha, Et Al., 1998; Organisciak, Et A. 2006, Andley, 2007), Parthasarathy, Et. Al., 2011). Fish Are The Oldest And Most Diverse Group Of Vertebrates Because Of The Lack Of Corneal Refractive Power In Water. Fish Lenses Have A Significantly Higher Refractive Index Compared To That Of Land Vertebrates. The Dominant Fish Lens Proteins Γm-Crystallins, Are Thought So Be Particularly Adapted For Dense Packing In These Hard Lenses (White, Et. Al., 1989; Slingsby, 1985). Alignment Of A □ And Γ With Other Known Crystallin Sequences Indicates That Fish Γ M-Crystallins From A Distinct Group That Differs From Those Of Mammals By Lacking Conserved Tryptophan Pairs In Each Domain And Possessing A Very High Metheonine Content (Pan, Et Al., 1994; Kiss And Cheng. 2008). The Main Optical Properties Of Lens And Comea Are Transparency And Refractive Power Proteins That Contribute To These Optical Properties Are Collectively Called Crystallin (Piatigorsky. 2007).

Material and Methods

The Fishes, Puntius Ticto (Hamilton, 1822) And Rasbora Daniconius Were Gathered From Feathers And Fins Aquarium Chh. Sambhajinagar And Kept Up In The Laboratory . Puntius Ticto And Rasbora Daniconius. The Eye Lens Putted In 0.05m Tris Hcl Of Ph 7.5 And Homogenized In Glass Homogenizer. The Methodology Adjusted Were As Portrayed By Kibbelaar And Bloemendal, (1975). Eye Lens Are Homogenized In 0.05 M Tris-Hci Support At Ph 7.5, Centrifuged At 15,000 X

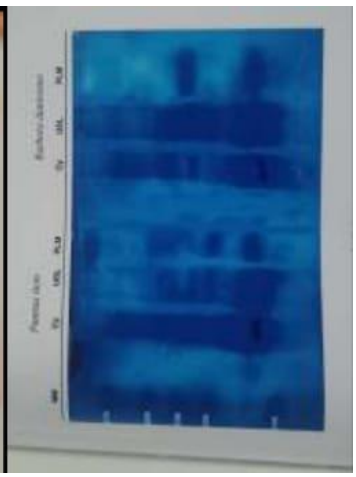
G. Also, The Pellet Was Washed Completely With The Goal That The Last Wash Doesn't Contain More Material Retaining At 280 Nm Than 0.05 Mg/Ml. The Last Pellet Is Suspended In 0.05 M Tris- Support Containing 6m Urea, 0.05m Nac1, And 0.001 M Edta, And Is Then Changed In Accordance With Ph 8.6. The Suspension Is Blended For 2 To 3 Hr. All Work Were Done At 4°C. From There On The Arrangement Was Centrifuged 20000xg For 150 Minutes. The Supernatant Contains The Usl Part. Planning Of Focal Point Plasma Films: The Urea-Insoluble Part Of Fish Focal Point Contains A Lot Of Plasma Layers, Containing Regular Characteristic Proteins. For The Readiness Of The Focal Point Plasma Film Part Two Systems Were Followed, Which Are Summed Up In Figures 1. The Primary Technique, Basically Restricted To Water And Urea Extraction, Depends Only Upon The (In) Dissolvability Properties Of The Layers. In The Second Strategy Both Thickness And Dissolvability Properties Of The Membranous Material Are Investigated. Arrangement Of Focal Point Dissolvable Protein: The Focal Point From These Fishes Were Analyzed In Chilled 0.05m Tris Hcl Support Ph 7.5 And Homogenized In Glass Homogenizer. The Systems Adjusted Were As Depicted By Kibbelaar And Bloemendal (1975). Around 10 Concentrates Were Acquired For Every Focal Point. Each Concentrate Was Centrifuged At 11000xg For 20minutes At 4°C To Isolate Water Solvent Protein From Water And Urea Insoluble Parts Displayed In Figure 1. Protein Content Of The Dissolvable And Insoluble Not Set In Stone By Uv Spectrophotometer At 280nm, Involving Bsa As Standard. All The Estimation Was Completed In Sets Of Three. The Examples Were Exposed To Sds - Page In 7.5% Gel And Run For 2 Hours Alongside Standard Sub-Atomic Markers As Portrayed By The Technique For (Laemmli, 1970).

Planning Of Focal Point Cortical Proteins: Focal Point Cortices Are Homogenized In 0.05m Tris- Hci Cushion At Ph 7.5, Centrifuged At 15,000 X G And The Pellet Was Washed Completely So The Last Wash Doesn't Contain Material Engrossing At 280nm More Than 0.05mg/Ml The Last Pellet

Was Suspended In 0.05m Tris-Support Containing 6m Urea. 0.05m Nacl And 0.0011m Edta And Changed In Accordance With Ph 8.6. The Suspension Was Mixed For 2 To 3 Hrs. All Work Were Completed At 4°C. The Supernatant Contains The Usl Division. Sds-Polyacrylamide Gel Electrophoresis: Dissolvable Crystallin Protein, Urea Solvent Focal Point Protein And Plasma Layers Protein Are Examined With The Sds-Polyacrylamide Gel Electrophoresis (Sds-Page), Sds-Page Was Performed Utilizing Microkin Unit, Techno Source (Mumbai), Crystallin Protein (10-20µg Per Test) Was Stacked On A Spasmodic Sds Polyacrylamide Gel (4% Stacking Gel, 7.5% Settling Gel) And Electrophoresed For 2 H At A Steady Voltage Of 150 V. Gels Were Stained With 0.1% Coomassie R-250 In 10% Acidic Corrosive, 40% Methanol And Destained With 10% Acidic Corrosive, 40% Methanol Tests From Every Arrangements, Depicted Above Were Exposed To Sds- Page. The Standard Sub-Atomic Markers (Medium Reach 97-14.3kda) Were Utilized As Provided By Merck, India. Protein Content: The Fish Eye Focal Point Were First Devicerated And Fish Focal Point Was Eliminated On Aluminum Foil Put On Ice In Ice. Fish Focal Point Weighing 100mg Were Homogenized In Chilled Sodium Phosphate Cushion (0.01m, Ph 7), Trailed By Centrifugation At 10000xg For 10 Minutes At 4°C. The Supernatant Protein Fixation In Eye Focal Point Tissue Was Examined At 280nm Utilizing Uv-Vis Spectrophotometer (Systronics 117). The Examples Were Weakened To 1:10 Proportion Prior To Taking The Perusing.

Result

Protein Content: The Protein Concentration In Muscle Was 0.092g/Ml And 0.069g/Ml Respectively. In The Present Study The Fish Lens Protein Were Divided Into Soluble Fraction Containing Crystalline, An Insoluble Fraction Containing Crystalline And Cyto-Skeleton Proteins, The Insoluble Fraction Was Further Analyzed For Protein By Using Urea Soluble Fraction And Plasma Membrane Fractions. Sds-Page Pattern Of Lens Proteins Of Fishes Is Shown In Plate. The Crystalline In (P.Ticto And R.Daniconius) Showed Four Bands In Sds-Page Viz.70,38,29, And 13.5kda.



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, □ 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 mostly, the oxido-reductase, and are often called as enzyme crystalline

(Piatigorsky and Wistow, 1991). Such a crystallin have 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 polypeptidedes in the B-crystallin of various mammalian and sub mammalians Among these the most common polypeptide are two with apparent molecular weight of 26K and 28 KDa. The 26 KDa polypeptide appear to be same as the poplypeptide designated BBp by (Herbrink and Bloemendal, 1974), later investigators reported the molecular weight of 27KDa major molecular weight common to both the BH and BL crystallin. A similar protein was reported in Guinea pig by (Huang et al., 1987). In the present study the crystalline proteins of P.ticto showed three bands 70, 38 and 29KDa and R. daniconius having six bands of 74, 43, 34, 27 and 22KDa respectively. We observed a relative abundance of two polypeptides, 27kDa and 22kDa in R. daniconius and P.ticto a 29kDa polypeptide. it appears that the accumulation tendency of 22kDa polypeptide may be either with age or changes in Na" or K ratio's. Though in the present study age was not considered, therefore, we cannot draw conclusion, but rather speculate, since the 27 and 29 kDa polypeptide occur together as subunits of ß3 subunits, there changing ratios suggest that they can assemble in different proportion to make up the B-crystallin molecules in a manner analogues to iso enzymic structure. Jiang et al., (1989) found 28KDa as a non glycosylated band in frog and tadpole lenses and it corresponds to □-crystallin. Jiang et al., (1989) speculated that this 28KDa may correspond to bovine lens BBP crystallin, which is similar to main B-crystallin in bovine lenses. In the present study we believe that the 29 and 27kDa may represent the B-crystallin as suggested y Jang et al., 1989). The (P.ticto and R.daniconius) 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.

Conclusion

Thus, we conclude that, though both the fishes belongs to Cyprinidae family, the protein derived from eye lens showed a 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.

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