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DISTRIBUTION AND PATTERN OF WATER-SOLUBLE PROTEINS OF LENS AS REVEALED BY GEL FILTRATION CHROMATOGRAPHY IN FISH *(CATLA CATLA)* OF DIFFERENT AGE GROUPS

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The water soluble proteins of the lens collected from different age group of the fish *Catla catla* were subjected to Sephadex G 200 gel filtration to fractionate and to characterize the protein and further to determine the elution volume (V_e) of different fractions. The relative proportion of each fraction was also calculated. Total number of fractions F_1 to F_4 was discernible through gel filtration. The molecular weight of these varied from 35,000 Da to 640,000 Da. Three subfractions were also noted in F_1 fraction. Interesting observations were, presence of α, β_H, β_L crystallins in all age groups and LMW proteins in older age group. α-crystallin present in major amounts in comparison to β_H & β_L crystallins. Decrease of α-crystallins (except in age group II) with the increase of age, counter balanced by the increase of β_H crystallins. Increase of β_H crystallins was followed by the decrease of β_L crystallins. Results suggest that proportionate distribution of different classes of proteins determined by column chromatography is widely variable.

Keywords: Lens – water soluble proteins – gel filtration – fish – aging

INTRODUCTION

Interest in the water proteins from different vertebrate group of lenses has increased considerably. The lens contains all the proteins synthesized in the lens during the whole life of the animal. Lens development and differentiation are the results of many developmental steps and/or tissue interactions. During differentiation, epithelial cells transform into lens fibre cells and through steps it becomes filled with distinctive collections of proteins. The lens proteins (crystallins) mainly as α, β, γ and δ have been identified through their molecular weight, chemical nature, isoelectric point and also the relationship with the immunology. This water soluble lens proteins have been well characteristed in human, in cattle [14, 28], in chick [6–8]. In addition lenses of some avian species possess δ crystallins (FISC) [8]. McDevitt [17] reported the pesence of three classes of crystallins as α, β and γ in *Rana pipens*. In a comparative study on several amphibians using electrophoretic techniques, McDevitt and Collier [18] suggest the presence of only three classes of crystallins. Various authors

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detected β and γ crystallins in human lens, α crystallins in lens epithelial cells and α, β, γ crystallins in elongating fibre cells of rat lens using different techniques [16, 22]. In some *Rana* and *Triturus* sp. however, two or three differently charged α crystallins are distinguished by immunoelectrophoresis or column chromatography [10]. Very little work has been done in fish lens. For the fish lens particularly *Catla catla,* the exact life span of the animal is not known. This is highly commercially important cultivable fish in India and 1 or 2 years old fish have a high value in the market. Though the natural population of *Catla catla* in some large rivers sometimes attains body weight body weight of 25 Kg or more within 20–22 years of their age, no scientific data are available on the life span and survival of this fish, because they are harvested much prior to their natural aging death. This sp. has been selected in the present study not only because of its commercial importance, but also for its high nutritive value and delicacy. Present study therefore is and endeavour to characterize the water soluble protein fraction through Sephadex G 200 gel filtration chromatography, in the lens of fish *Catla catla* in dependence on age.

MATERIALS AND METHODS

Fish *(Catla catla)* lenses were collected from different age groups of fishes that have a high demand in the market, ranging from 25 to 550 days. Accordingly, six groups were made depending upon different age as age group I through age group VI as per following schedule.

Age groups	I	II	III	IV	V	VI
Age in days	25–35	60–85	150–180	240–280	360–400	490–550

Each group consisted of 15 to 20 animals and 30 to 40 lenses were collected for one group depending on the age groups and the quantity of the materials required for the experiment.

The proteins in the lens were studied qualitatively through the separation of the components by their molecular weight in Sephadex gel filtration. 300 mg of lens protein in 2 ml of the buffer were loaded on the top of the prepacked Sephadex gel column (50×2.5 cm). Three ml fractions were manually collected and the flow rate was maintained at 12 ml/hour.

Void volume (V_0) *determination*

A definite amount of the dextran sample was applied to the column and the absorbancy of collected material was measured at 625 nm in a spectrophotometer (LKB, Stockholm). The void volume of the column was determined by measuring the volume of the blue dextran collected from the point of sample application to the center of effluent peak.

Elution volume (V_e) *determination*

Low and high molecular weight gel filtration calibration kits (Sigma, USA) consisting of thyroglobulin (669,000 Da), ferritin (440,000 Da), catalase (232,000 Da), aldolase (158,000 Da), bovine serum albumin (66,000 Da), ovalbumin (43,000 Da), carbonic anhydrase (29,000 Da) and cytochrome C (12,400 Da) were used as standard protein. The protein standard was applied to the column in equal volume as used for blue dextran void volume determination. Absorbance of the eluted standard portein was measured at 280 nm and the elution value of the protein standard was determined by the same procedure followed for the void volume determination.

Molecular weight determination of eluted fraction

Molecular weight of eluted fraction was determined by the same method as applied to the standard protein. V_e/V_0 for each protein fraction was calculated and their molecular weight was determined from the prepared standard curve (stoke radius) by plotting V_e/V_0 versus molecular weights of each protein.

Statistical methods

Statistical analysis was performed using one way analysis of variance (ANOVA). A value of P less than 5% was considered as significant.

RESULTS

The Sephadex G-200 elution patterns for soluble fish lens homogenate of different age groups show three fractions separated from youngest group of lenses (age group-I and -II) (Fig. 1). The first peak (F_1) from the column contains primarily a high molecular weight protein while the last protein peak (F_3) of these two age groups has a molecular weight of 92,000 Da. Similarly calculated, the molecular weight for the first peak is 615,000 Da for both the age groups. The first and the second age groups are similar regarding molecular weight of all the fractions. The second peak (F_2) contains protein of molecular weight 200,000 Da. It is also evident from Table 1 that the ratio of V_e/V_0 for different peaks observed is not same and indicates change in molecular weight for different fractions. When comparison of elution patterns are made between these two age groups, the first fraction has the highest peak, and also the second fraction shows the highest peak than the third-one.

The relative proportions for the three fractions of the age group-I show the values of 49%, 29% and 22% whereas the age group-II shows the value of 48%, 30% and 22% (Table 1).

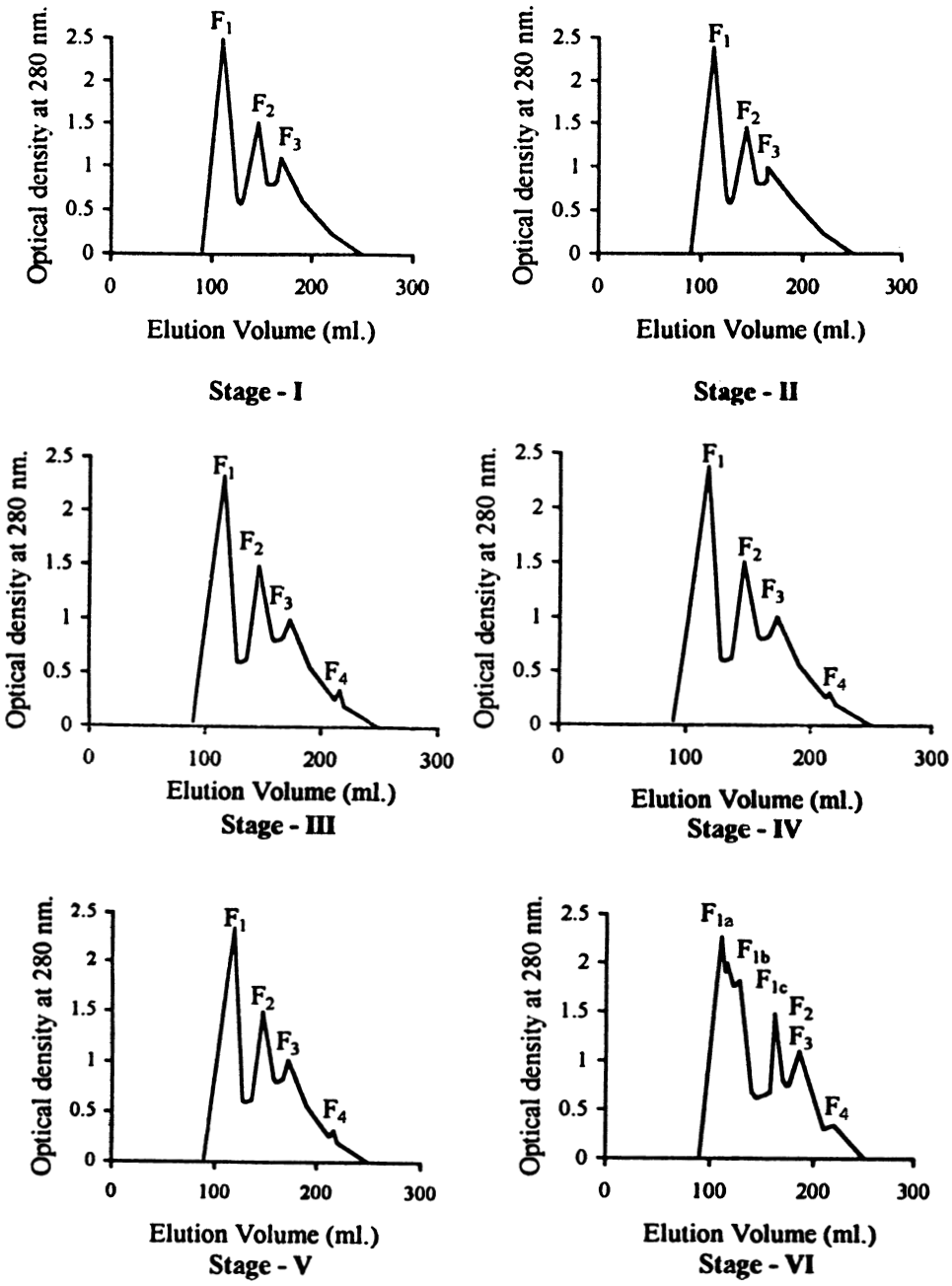

Fig. 1. Elution pattern of water soluble lens extract of fish *Catla catla* on Sephadex G-200 column (50 cm×2.5 cm)

The elution pattern for age groups III, IV and V show four fractions as F_1, F_2, F_3 and F_4 (Fig. 1). The F_1 fraction shows a molecular weight of 600,000 Da for both age groups IV and V but for age group-III, the molecular weight is 615,000 Da. The calculated V_e/V_0 for F1 of these three age groups are 1.17 for age group-III and 1.19 for age groups IV and V. The last protein peak F_4 of age groups III and IV contains low molecular weight (LMW) components of the same molecular weight 35,000 Da. But the fourth peak of age group V contains slightly higher than the other two groups. The molecular weight of F_2 varies among these three age groups. But the other franction F_3 does not show similar molecular weight. Variation is also observed in the ratio of V_e/V_0 for different protein peaks. In age groups III, IV and V, the first fraction (F_1) shows the highest peak than the other, while a peak of small height is observed in the fourth (F4) fraction.

Table 1
Characteristics of water soluble lens protein component in different age groups of fish *Catla catla* separated by Sephadex G-200 gel filtration

Age groups	Gel filtration components						
I	Fraction No.		F_1		F_2	F_3	
	V_e/V_0		1.17		1.63	1.91	
	Molecular wt.		615,000		200,000	92,000	
	Relative propn.		49%		29%	22%	
II	Fraction No.		F_1		F_2	F_3	
	V_e/V_0		1.17		1.63	1.91	
	Molecular wt.		615,000		200,000	92,000	
	Relative propn.		48%		30%	22%	
III	Fraction No.		F_1		F_2	F_3	F_4
	V_e/V_0		1.17		1.63	1.91	2.29
	Molecular wt.		615,000		200,000	92,000	35,000
	Relative propn.		47%		30%	20%	3%
IV	Fraction No.		F_1		F_2	F_3	F_4
	V_e/V_0		1.19		1.65	1.91	2.29
	Molecular wt.		600,00		190,000	92,000	35,000
	Relative propn.		46%		30%	20%	4%
V	Fraction No.		F_1		F_2	F_3	F_4
	V_e/V_0		1.19		1.68	1.91	2.23
	Molecular wt.		600,000		185,000	92,000	45,000
	Relative propn.		45%		31%	20%	4%
VI	Fraction No.	F_{1a}	F_{1b}	F_{1c}	F_2	F_3	F_4
	V_e/V_0	1.10	1.28	1.34	1.70	2.00	2.23
	Molecular wt.	640,000	485,000	425,000	180,000	78,000	45,000
	Relative propn.	41%	4%	3%	27%	19%	6%

The ANOVA test shows that the variation in molecular weight in F_1 fraction when considered all six age groups together is not significant ($F_{(5,18)} = 0.222$; $P > 0.05$). But comparison amongst three categories comprising of age-groups I, II, III (615,000 Da), IV, V (600,000 Da) and VI (640,000 Da) in terms of molecular weight distribution reveal a significant difference ($P < 0.05$). However, molecular weight in F_2 fraction does not show any significant ($F_{(5,18)} = 0.217$; $P > 0.05$) variation in any case. A high significant difference ($P < 0.001$) is observed in the molecular weight distribution in F_3 fraction between age groups VI (78,000 Da) and the rest age groups (92,000 Da). Similarly, F_4 fraction of age groups III and IV (35,000 Da) also differs significantly ($P < 0.05$) from age groups V and VI.

DISCUSSION

Different authors use various isolation procedures to know the proporties of lens proteins. Studies on the water-soluble proteins from fish lenses have been few [8]. Most of the author employed urea or SDS-PAGE [23, 29] to detect the distribution of high molecular weight water-soluble proteins. Information is mainly available showing great differences for whole lens or lens part derived from electrophoretic study. Using agarose gel electrophoresis Francois & Rabey [12] detected thirteen separate soluble proteins in human newborn lens and as many in other mammalian species. But three fractions are noted in bovine lens of different ages through continuous flow electrophoresis [11]. The present study of gel filtration with Sephadex G 200 material for convenient separation of water soluble proteins as separated through Sephadex give a picture that the fish lens contains as many as six components in older age group and no less than three components in earlier age group of the lens. This suggests that the higher age groups have a novel protein fraction, where as the earlier groups (I & II) there is no protein of low molecular weight. This is a clear indication of variation in protein composition in aging fish lens.

Though the immunological reaction for a specific class of proteins could not be performed, yet there was indication that F_1 fraction of water soluble proteins of the fish lens eluted on Sephadex gel filtration is α-crystallin. Since, the protein of F_1 fraction of the WS protein has the molecular weight range of 425,000 Da to 640,000 Da, even the molecular weight of α-crystallin of some mammalian species ranges from 400,000 Da to 1,200,000 Da in bovine lens [13], 575,000 Da in rabbit lens [3], 640,000 Da in fish Tilapia [15], it can be reasonably assumed to be α-crystallin. It is the α-crystallin [9], having V_e/V_0 value ranging from 1.10 to 1.34 in our present experiment. Present study also shows that the α-crystallin comprise about half of the total lens crystallin in the fish lens. The proportion of α-crystallin is known to vary even within a given class e.g. 11% to 30% in adult mammals [9], in juvenile mammals it is as high as 54% [27] and 39% to 42.5% in fish Tilapia [15]. Our values of 41% to 49% in fish lens agree with the values reported in literature. When F_2 fraction of all age groups are considered it shows a molecular weight range of 180,000 Da to 200,000 Da. Thus, it may well be β_H crystallin because its molecular weight is

comparable with the molecular weight range of 150,000 Da to 200,000 Da of other vertebrate species [4, 5, 20, 21, 24, 25]. Similarly the molecular weights of β_H crystallins of different mammalian species (rabbit & calf) ranges between 165,000 Da and 200,000 Da and 190,000 Da in chicken [2]. Zigler and Sidbury [26] reported that the V_e/V_0 ratio of β_H fraction separated on G-200 gel filtration in the blue fish is 1.34 and in *Calotes* it is 1.47 [19]. The V_e/V_0 for F_2 in fish *Catla* ranges from 1.26 to 1.41 (V_e/V_0 was computed using the elution volume for α-crystallin as the void volume) which suggests that F_2 may well be β_H crystallin.

In addition, it is interesting to know that the presence of another fraction (F_3) with molecular weight ranges of 78,000 Da to 92,000 Da in the present study and V_e/V_0 ratio ranges between 1.91 to 2.00. The β_L crystallin has a molecular weight 80,000 Da in calf, 73,000 Da in rabbit and 75,000 Da in rat lenses [2]. It is also found that β_L crystallin varies from 50,000 Da to 80,000 Da in different parts of the bovine lens [1]. The V_e/V_0 ratios for *Rana, Calotes* and *Gallus* are 1.96, 1.85 and 1.82, respectively. The V_e/V_0 for this fraction in the present study of fish lens shows value of 1.74 to 1.82 (considering α-crystallin as void volume) which is comparable with the V_e/V_0 of blue fish and chicken ($V_e/V_0 = 1.91$), leopard frog ($V_e/V_0 = 1.89$), turtle ($V_e/V_0 = 1.76$) [26]. These help to infer this fraction as β_L crystallin.

The last fraction F_4 for age groups III to VI possesses a molecular weight of 35,000 Da to 45,000 Da. This fraction appears to be a low molecular weight (LMW) on the basis of the reported value of the calf lens around 50,000 Da [4, 25]. According to Zigler and Sidbury [26] the frog lens contains an unclassified crystallin of a molecular weight near 37,500 Da, but Bindles et al. [2] reported a molecular weight of 40,000 Da in the same species. The present study shows V_e/V_0 for this fraction ranges from 2.23 to 2.29 which is comparable with the V_e/V_0 value of cattle (2.12) [19], of blue fish (2.27), of turtle (2.38) [26]. Considering the molecular weight and V_e/V_0 value of this fraction, it can be designated as low molecular weight protein. It can be concluded from the observations that with the differentiation of the lens the distributions and patterns of lens crystallins determined by gel filtration chromatography are widely variable.

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