

Cataractous lens and its environment

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Abstract

Senile cataract is responsible for significant visual impairment in aged people worldwide. Several risk factors are generally accepted which may lead to the loss of lens transparency. These include diabetes, malnutrition, drugs, radiation and diarrhoea. Transparency of the lens depends on unique arrangement of tightly packed fibers which in turn rely on certain structure. These proteins, the crystallins, are thus responsible for establishing remarkable optical properties of the lens. The role of crystallins in the process of opacification requires a detailed study of their structure, environment, mode of interaction among each other and individual and collective behavior in cataractogenesis.

Present communication provides an update of our research on senile cataract lenses including studies on aqueous humor, comparative profile on different stages of cataract development and structural studies on crystallins.

INTRODUCTION

Cataract, the lens opacity, is the major cause of visual impairment throughout the world (ref. 1). About 40 % of the estimated 42 million blind people worldwide are due to cataract with 13 million in developing countries (ref. 2). Cataract in the region is responsible for 81 % of the total blindness in India (ref. 3), 72 % in Nepal (ref. 4), 72 % in Pakistan (ref. 5) and 26 % in China (ref. 6). In Pakistan and India, incidence of cataract is higher and onset of cataract is 20 years earlier than in Europe (ref. 5,7). Surgical removal of the lens is the only remedy at present, however, it is not a perfect solution of the problem as besides being expensive, it may lead to other ocular damages (ref. 4). Development of nonsurgical procedures require a much deeper understanding of the structure and metabolism of the lens.

Lens is an avascular tissue surrounded by anterior/posterior chamber and vitreous body which contain aqueous and vitreous humor respectively. The biochemical composition of aqueous humor in human eye has always been a matter of great interest for elucidating the pathogenesis of all types of cataracts, as nutrition of living lens completely depends on this fluid (refs. 8,9). Optical properties of the lens depend on diverse group of lens specific structural proteins, called crystallins (refs. 10,12). High concentration of these proteins provide medium of high refractive index necessary for proper functioning of the lens. Irregularities in refractive index may induce light scattering as often found in certain types of cataract (ref. 13). In mammalian lenses, crystallins account for 90 % of the soluble proteins which belong to two superfamilies: α and $\beta\gamma$. α -crystallins are derived from two genes (αA , αB) while $\beta\gamma$ -crystallins are encoded by at least thirteen genes. In avian and some reptilian lenses, γ -crystallin is replaced by δ -crystallin. Crystallins have been studied extensively; their primary structures elucidated from various species, and three dimensional structure of some also determined (refs. 4,11).

Among various types of cataract (senile or age related) accounts for the vast majority, in which number of other factors superimpose normal aging process. The initiation and evolution of cataract is multifactorial and as cataract progression is very slow it is difficult to identify the initial causes of cataractogenesis. However various risk factors can be listed which may lead to the loss of lens transparency. These include diarrhoea, malnutrition, genetic and metabolic disorders, intraocular

diseases, exposure to chemicals and radiations, diabetes, drugs and old age. Although it is difficult to identify initiating cause(s) and precataractous alterations, understanding of lens structure, metabolic processes and related fluid (aqueous humor) may represent the changes induced due to opacity which in turn might help in determining preventive measures.

Present communication deals with the work carried out in our laboratories on different aspects of cataract formation. These include studies on aqueous humor, different stages of cataract development and sequence determination of a crystallin.

MATERIALS AND METHODS

Aqueous humor was aspirated during cataract surgery and stored at -30°C till further analysis. Aqueous humor was centrifuged at $10,000 \times g$ at 4°C for 20 minutes supernatant was used for all studies.

Cataractous lenses were obtained from Rehmatullah Benevolent Trust Eye Hospital, Karachi, and classified according to Pirie (ref. 14). Lenses were homogenized in deionized water and centrifuged for 20 minutes at $20,000 \times g$ at 4°C . Supernatant was collected, lyophilized and stored at -30°C till further analyzed. Clear cadaver lenses were a donation by the Lions Club, Pakistan.

Crude lens samples were fractionated on Sephadex G-50 (Superfine). Rechromatography of low molecular mass protein was carried out on Vydac C4. Different types of lenses (immature, mature, hypermature, cataract nigra and clear) were fractionated on RP-HPLC using Vydac C4 with 0.1 % TFA and acetonitrile gradient. SDS-PAGE was performed on 12.5% gel according to Laemmli (ref. 15). Sequence studies of purified protein were carried out by automated gas phase sequencer (470 A, Applied Biosystems).

RESULTS AND DISCUSSION

Comparison of amino acid composition from aqueous humor of Pakistani subjects with European (ref. 16) and American (ref. 8) data revealed (table 1) that most of the amino acids except glycine were in lower concentration in local population. In addition, levels of proline and citrulline were higher than in American population. The variation in the composition probably reflects different dietary habits in different population.

Cataractous lenses have been classified into four groups (immature, mature, hypermature and cataract nigra) according to their colour and opacity. Fig. 1a-e represent HPLC profiles of different

Table 1: Free Amino acid contents in Aqueous humor of Pakistani, European and American subjects with Senile Cataract. The results (mmoles/l) are Mean \pm S.E.

Amino acid	Present Study	European Study	American Study
Threonine	0.098 \pm 0.011	0.119 \pm 0.005	0.150 \pm 0.007
Serine	0.066 \pm 0.009	0.142 \pm 0.005	-
Glutamic acid	0.012 \pm 0.001	0.038 \pm 0.004	0.010 \pm 0.001
Proline	0.037 \pm 0.005	-	0.020 \pm 0.002
Citrulline	0.012 \pm 0.005	-	0.005 \pm 0.001
α -Amino butyric acid	0.016 \pm 0.006	0.025 \pm 0.001	0.030 \pm 0.002
Valine	0.105 \pm 0.017	0.292 \pm 0.013	0.340 \pm 0.008
Isoleucine	0.021 \pm 0.002	0.055 \pm 0.003	0.050 \pm 0.004
Leucine	0.051 \pm 0.009	0.135 \pm 0.006	0.140 \pm 0.005
Tyrosine	0.028 \pm 0.006	0.085 \pm 0.003	0.110 \pm 0.005
Phenylalanine	0.052 \pm 0.008	0.089 \pm 0.003	0.110 \pm 0.005
Glycine	0.018 \pm 0.001	0.019 \pm 0.001	0.020 \pm 0.003
Alanine	0.138 \pm 0.014	0.265 \pm 0.012	0.340 \pm 0.017
Phosphoserine	0.041 \pm 0.003	-	-
Taurine	0.042 \pm 0.006	-	0.050 \pm 0.007
Ornithine	0.015 \pm 0.003	0.027 \pm 0.002	0.030 \pm 0.002
Lysine	0.031 \pm 0.003	0.164 \pm 0.008	0.150 \pm 0.006
Histidine	0.054 \pm 0.010	0.067 \pm 0.003	0.080 \pm 0.004

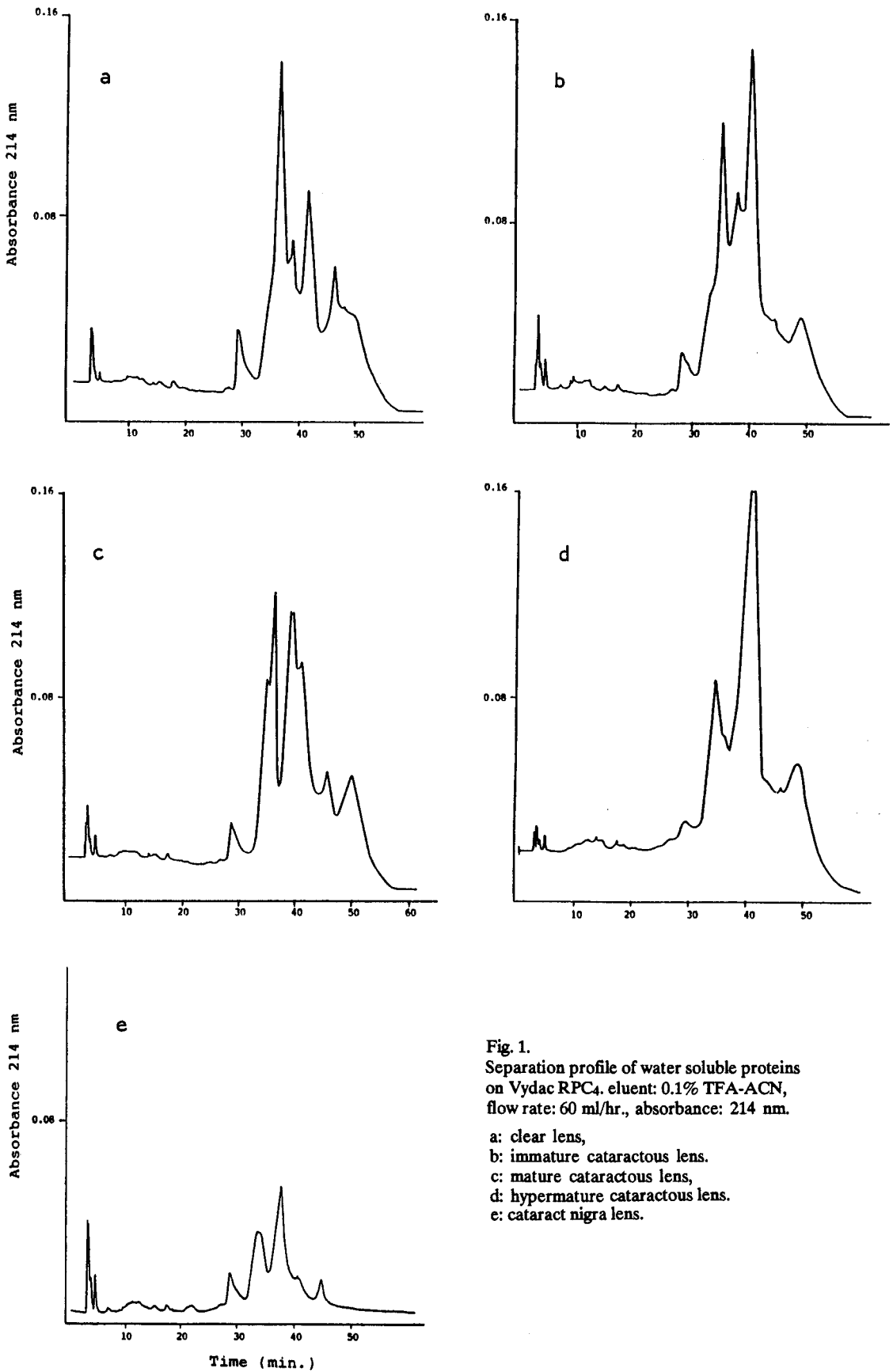


Fig. 1.
 Separation profile of water soluble proteins
 on Vydac RPC4, eluent: 0.1% TFA-ACN,
 flow rate: 60 ml/hr., absorbance: 214 nm.

- a: clear lens,
- b: immature cataractous lens.
- c: mature cataractous lens,
- d: hypermaturing cataractous lens.
- e: cataract nigra lens.

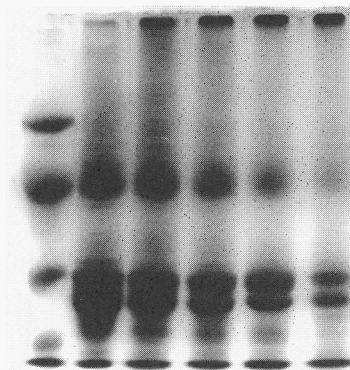


Fig. 2.
SDS polyacrylamide gel electrophoresis of water soluble lens crystallins from different types of human lenses.

From left to right: 1, mol. mass std; 2, clear; 3, immature; 4, mature; 5, hypermature; 6, cataract nigra.

types of cataractous and clear (cadaver) lenses. The separation profile of water soluble fractions from different types of cataractous and clear lenses showed four-five peaks depicting clear changes in peak areas. SDS-PAGE of crude water soluble lens crystallins from different stages of cataract when compared with clear (cadaver) lenses (Fig. 2) revealed gradual loss in concentration of crystallins with the progression of opacity, confirming earlier reports that solubility decreases with the maturity of cataract (ref. 14). Our results also suggest that earlier assumption of decrease in only low molecular mass proteins (especially γ -crystallins) during cataract progression is not true and need to be revised, as all types of crystallins decrease with cataract progression, though in different proportion.

We have succeeded in isolation, purification and elucidation of primary structure of human γ s (formerly β s) crystallin (ref. 17). Purified protein has a molecular mass of 20,891 Da with 177 amino acid residues having acetylated serine at the N-terminal (Fig. 3). Our studies reveal that human γ s is much closer to members of γ -family as compared to β -family as has been observed for bovine (ref. 18). Members of $\beta\gamma$ superfamily have four consecutive Greek Key motifs arranged in two compact domains joined by a connecting peptide (refs. 19-21). The N terminal domain contains motif 1 and 2 while C terminal domain contains motif 3 and 4. Solvent facing motifs are 1 and 3 while remaining two motifs (2 and 4) form interdomain region. Certain key residues appear to be essential

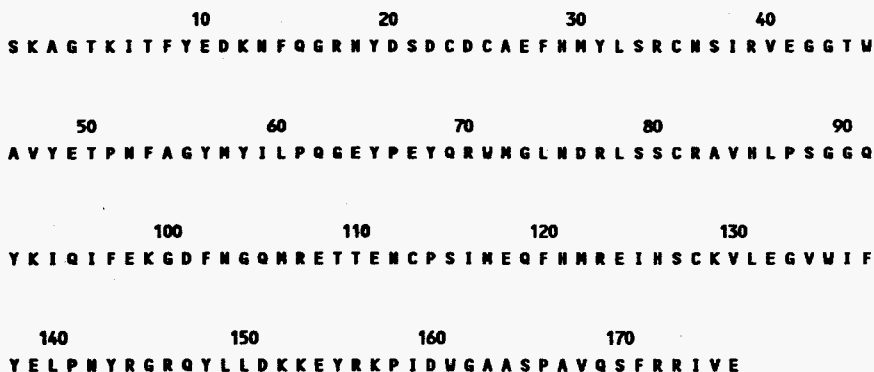


Fig. 3. Primary structure of human γ s-crystallin

for this particular fold which include Tyr6, Glu7, Phe11, Gly13 and Ser34 in motif one (and their equivalents). It has been suggested that all γ (and β) crystallins which contain these residues may adopt same tertiary fold (ref. 22). Recent studies have, however, shown that although β and γ -crystallins have similar motifs within the domains, their conformations are different (ref. 23). The

two domains in β B2 crystallin are not in close contact with each other as in γ B-crystallin and are separated by an extended connecting peptide. It is inferred that the sequence differences in the connecting peptide of β and γ -crystallin are primarily responsible for the different conformation. All γ -crystallins contain a Gly residue at position 86 in the connecting peptide whereas all β -crystallins studied so far contain amino acids with bulky polar side chain as residue in this position. It is logical to suggest that the presence of Gly86 in γ B-crystallin allows the connecting peptide to take a sharp turn so that the two domains come closer and interact with each other. In the present study, human γ S-crystallin does contain Gly residue at the same position suggesting that it should have a similar conformation as γ -crystallins, but has only 54 % sequence identity with γ crystallins. It is rather low as compared to 79 % identity amongst the members of γ family. The human γ S crystallin has an extended connecting peptide with Gly preceding Gly 86 and also an extended N terminal arm with blocked N terminal residue which have similarities with the β crystallins. We, therefore, propose taking into account the above facts that it should be an intermediate between β and γ -crystallins originating from $\beta\gamma$ -superfamily.

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