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AGE DEPENDENT CHANGES IN THE ABUNDANCE OF THE MAJOR POLYPEPTIDES OF HUMAN LENS MEMBRANE

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SUMMARY: Changes in the abundance of the major polypeptides of human lens membrane with aging and cataractogenesis have been studied. It has been shown that with aging the relative amounts of the two major membrane polypeptides, 26,000 and 22,000 dalton components, change up to the age of approximately 40. Thereafter the relative abundance of these two polypeptides remains constant. Explanations for this alteration are discussed in relation to similar age dependent changes of the proteins of the human lens.

INTRODUCTION

The encapsulated avascular lens is a transparent tissue whose major function is to focus light upon the retina. It contains a single layer of epithelial cells on the anterior subcapsular surface which continually transform into fiber cells throughout life. As new fiber cells are formed the old cells are displaced toward the center of the organ. There is also pyknosis of the nucleus and loss of other intracellular organalles (1). Thus, the fiber cells present in the inner (nuclear) region of the lens represent cells formed in early life while the peripheral tissue is representative of newly synthesized fiber cells. Since little protein turnover is observed in the nuclear region of the lens (2), it is probable that the proteins in this region represent material produced at a young age. Therefore, both cell fiber structure and proteins are conserved throughout life in this unusual tissue.

The abbreviations used are: HMW: high molecular weight; SDS: sodium dodecyl sulphate.

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It has been shown that the major soluble structural lens proteins, the crystallins, aggregate with aging forming HMW aggregates and insoluble components (3). It has been suggested that this process is related to cataract formation (4). Recently, a HMW disulfide linked aggregate has been isolated from cataractous lenses (5). These aggregates have been shown to contain an extrinsic membrane polypeptide of 43,000 daltons. It has been suggested that this polypeptide may act as an enucleation site for the formation of these disulfide linked aggregates (6). The unusual preservation of cell structure and molecular species with aging, as well as the interaction of membrane polypeptides with soluble lens constituents (6), has led to a study of the influence of aging on membrane structure.

Previously membrane rich fraction from the insoluble proteins was prepared by laborious washing with 6M urea (7). Recently new methodology has been developed in this laboratory for the preparation of pure lens membrane (8). With this method membrane from a single lens can be prepared with ease. In this communication the effect of aging and cataract upon the polypeptides of lens membrane prepared by this procedure is reported.

METHODS

Human lens membranes were prepared from normal individual lenses of known age as described previously (8). The material was homogenized in 7M guanidine HCl, 0.2M Tris, 2 mM phenyl methyl sulfonyl fluoride and treated with citraconic anhydride as previously described. All free sulphydryl groups were blocked by reaction with iodoacetamide in the above buffer at 37°C for 2 hours. Citraconic anhydride (50 fold excess assuming 20 lysine residues/20,000 dalton) was used to modify the ε NH_2 groups of lysine after alkylation reaction in the same buffer. SDS polyacrylamide gel electrophoresis of the membrane polypeptides were performed according to Fairbanks et al (9). Coomassie blue stained gels were scanned at 590 nm with a Gilford gel scanner interfaced with a DEC minicomputer for the purpose of integrating selected peak areas representing polypeptides of different molecular weights. The sum total of the two major polypeptides of 26,000 and 22,000 daltons was normalized to 100% and the percentage of each component was plotted.

Membranes were prepared for electron microscopy by fixation in 5% glutaraldehyde and postfixing them with 1% osmium tetroxide. Micrographs of sections from blocks were taken with a Philips 300 transmission microscope.

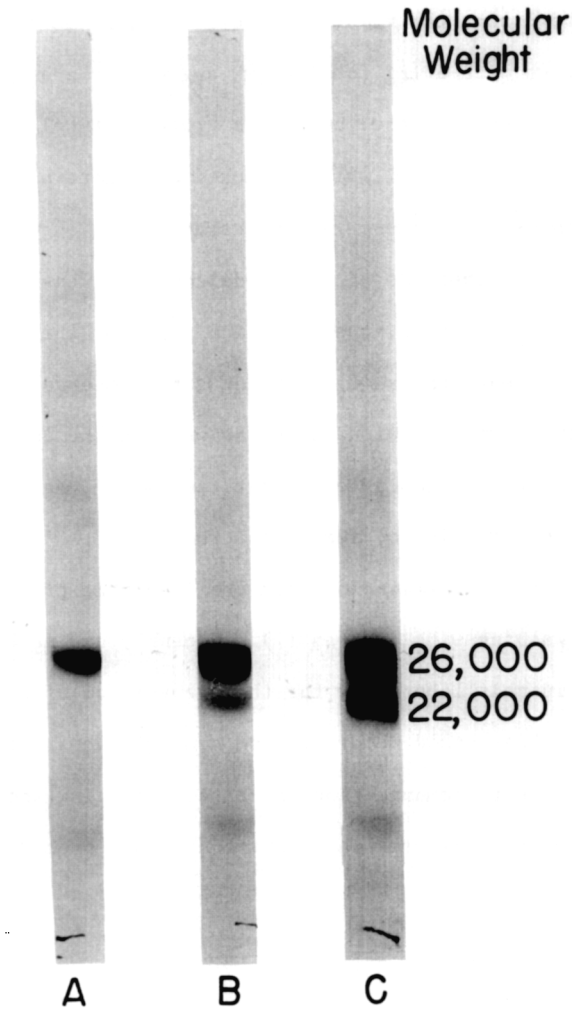

Figure 1. SDS polyacrylamide gel electrophoresis profile of membrane prepared from (A) 6 month old, (B) 18 year old, and (C) 55 year old human lens. The gels were calibrated by running standard citraconylated proteins.

RESULTS

As shown in Fig. 1, the SDS polyacrylamide gel electrophoreses of membranes prepared from a 6 month, 18 year and 55 year old normal human lens show a striking change in the abundance of the major polypeptides that are present in these membrane preparations. While the main intrinsic polypeptides of the young lens (6 month) have a molecular weight of 26,000, the older lens (18 and 55 year) membrane contains two major polypeptides of

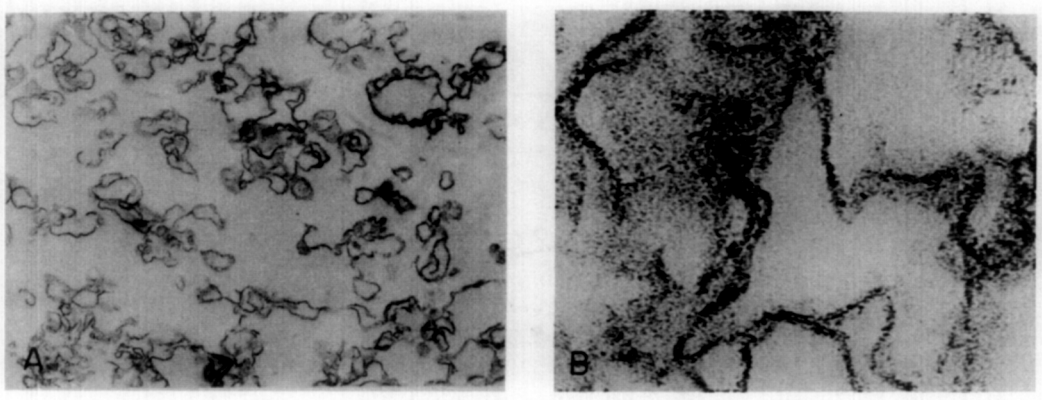

Figure 2. Electron micrographs of membrane obtained from a 55 year old human lens. (A) x 24,000, (B) x 170,000.

molecular weights of 26,000 and 22,000 (8,10). To ascertain that this observed difference in the polypeptides of human lens membrane is due to aging and not due to the presence of extraneous proteins, membrane preparations were examined by electron microscopy. Figure 2 shows two typical micrographs at low and high resolution. Each membrane preparation was subdivided and imbedded in different blocks, sectioned and micrographs were taken. In all these micrographs essentially no cell organelles or extraneous proteins were observed indicating a high degree of purity of the membrane preparation consistent with previous experience with this methodology (8).

To determine more quantitatively changes in the intrinsic polypeptides of lens membrane with aging, membrane from different aged normal lenses was isolated and the polypeptide composition determined by SDS polyacrylamide gel electrophoresis. The amount of 26,000 and 22,000 dalton polypeptides was determined by scanning the coomassie blue stained proteins and normalizing the abundance of the two polypeptides to 100%. Since the other polypeptides in these preparations are present in minor amounts and do not change in abundance with age, they have not been considered in this study.

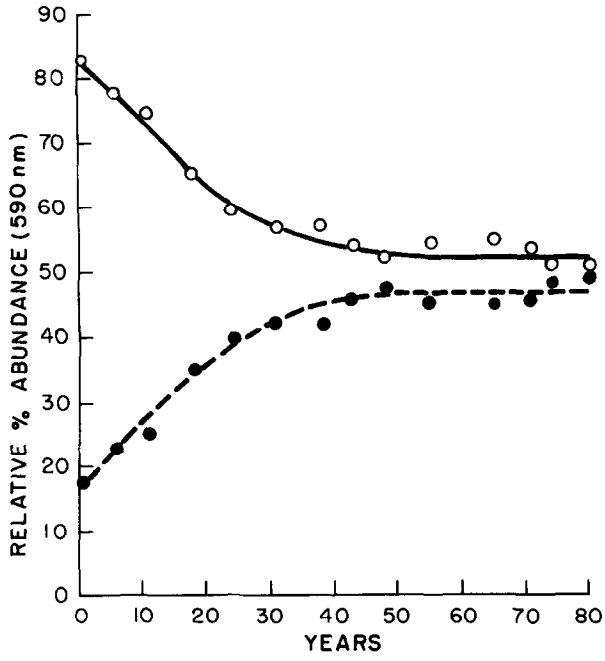

Figure 3. Changes in percentage abundance of the 26,000 (o) and 22,000 (●) dalton lens membrane polypeptides with the age of the lens.

Figure 3 shows the plot of the relative amounts of the 26,000 and 22,000 dalton polypeptides in different aged lenses. In young lenses approximately 83% of the two major membrane polypeptides is represented by the 26,000 dalton species. With increasing age the 22,000 dalton components become more abundant, increasing from 17% at age 6 months to 45% at 40 years. It is of interest to note that from approximately 40 years of age the relative amounts of these two polypeptides remain constant.

Since the nucleus, the inner region of the lens, is the oldest part of the tissue and is presumed to contain the fetal fibers, 60 year old human lenses were disected into nuclear and cortical sections. The relative proportion of the 26,000 and 22,000 dalton polypeptides in these two sections of the tissue was found to be approximately the same as that in the whole lens. Also, membranes isolated from cataractous lenses show the same ratios of these two polypeptides as that obtained from comparably aged normal lenses.

DISCUSSION

It is clear from Figure 3 that with aging the relative abundance of the 26,000 dalton polypeptides decreases and that of the 22,000 dalton polypeptides increases. This process continues up to the age of approximately 40. After that time the relative abundance of these two polypeptide species remains relatively constant. This change in relative abundance can be due to a number of factors. Conceivably with aging the lens fiber membrane may be altered to meet different physiological requirements thus synthesizing larger amounts of the 22,000 dalton polypeptides. However, such a situation is unlikely since very little protein synthesis occurs in the nuclear region of the lens(2). Furthermore, the abundance of the 26,000 and 22,000 dalton polypeptides is similar in both the inner and outer regions of the tissue. Preliminary studies with chelating agents indicate that at least part of the 22,000 dalton polypeptide may be bound to the membrane via divalent cation. This raises the possibility that perhaps all of the 22,000 dalton species represents a soluble component that has been associated with the membrane due to age dependent changes in the structure of these polypeptides, the membrane, or both. The lack of further accumulation of these polypeptides after the age of 40 may be indicative of an equilibrium between bound and free species.

A third explanation for this change in abundance of these two polypeptides is that proteolytic or photochemical degradation of the 26,000 dalton species occurs with aging generating the smaller polypeptides. Alpha crystallin A chain is known to degrade in vivo by proteolysis or photochemical reaction to a smaller polypeptide (11). Thus this route for the production of the 22,000 dalton components cannot be ruled out. However, it should be pointed out that unlike the 26,000 dalton polypeptides, the degradation of A chain does not attain an equilibrium at a certain age but continues throughout life (12). Further work to define the relationship of these two membrane polypeptides is in progress.

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