Human alpha-crystallin-III isolation and characterization of protein from normal infant lenses and old lens peripheries

Debdutta Roy and Abraham Spector

Alpha-crystallin isolated from the peripheries of old normal or cataractous lenses appears to be identical, consisting of eleven polypeptides, five B, and six A chains. In contrast, alpha-crystallin isolated from normal six-week-old human lenses has only three major polypeptides, corresponding to B, A, and A, of the old human lens protein as well as small amounts of some of the other components. Comparisons with bovine alpha-crystallin are also reported. Based on gel filtration experiments with Bio-Gel A-1.5m, two distinct populations of alpha-crystallin were found in old lens periphery, one containing species greater than 1.5 \times 10^9 daltons and another of approximately 9 \times 10^8 daltons. In the cataract preparations, the higher molecular weight fraction is predominant. This fraction is not present in young lenses.

Key words: lens, cataract, alpha-crystallin, aging aggregation, polypeptide chains.

Alpha-crystallin is one of the major structural proteins of the lens. In the bovine tissue, it has been demonstrated that with aging, this protein aggregates to high molecular weight (HMW) species greater than 50 \times 10^6 daltons and is also the major component of the so-called insoluble fraction. This transformation occurs primarily in the nuclear region of the lens. Newly synthesized (NS) alpha-crystallin is a physically homogeneous macromolecule of approximately 7 \times 10^5 daltons. It contains two different 2 \times 10^4 dalton polypeptide chains designated A and B, held together by noncovalent forces. Very shortly after formation, post-translational reactions produce a modified A polypeptide designated A and a modified B polypeptide designated B. Transformation to a heterogeneous macromolecular population with an increase in the weight average molecular weight (Mw) is concomitantly observed. In older lenses, additional changes in the A and B polypeptides have been reported as well as a shift to HMW aggregates and the formation of increasing amounts of the insoluble protein. Because of the possible relationship between the development of HMW and insoluble protein and cataract formation there is considerable interest in the structure of alpha-crystallin and its relationship to these macromolecular species.

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Table I. Amino acid composition of alpha-crystallins *

<table>
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<th></th>
<th>Old normal periphery</th>
<th>Old cataract periphery</th>
<th>Infant</th>
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<tr>
<td>CM Cys</td>
<td>11</td>
<td>10</td>
<td>13</td>
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<tr>
<td>Asp</td>
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<td>95</td>
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<td>Thr</td>
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<td>45</td>
<td>51</td>
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<td>Met</td>
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<tr>
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<tr>
<td>Lys</td>
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<td>His</td>
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<tr>
<td>Arg</td>
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*Results are expressed as residues per 1,000 residues. Values are not corrected for destruction during hydrolysis.

Recently, human alpha-crystallin has been characterized.13, 14 Based upon sedimentation equilibrium experiments the NS protein isolated from very young normal human lenses appears to be physically homogeneous and has a molecular weight of approximately $7.5 \times 10^5$.13 It contains three polypeptides, two presumptive A chains, and one presumptive B chain. It is not known at present whether both A chains are synthesized or if a rapid post-translational transformation causes the appearance of the second A chain.

Alpha-crystallin has also been isolated from old cataractous lens periphery.14 This preparation is physically heterogeneous with species ranging from less than $1 \times 10^6$ to greater than $5 \times 10^6$ daltons. A complex group of eleven polypeptides was observed. Although the cataractous lenses used for that study contained opacities with little pathology in the peripheral region, there is a question of whether alpha-crystallin obtained from old normal lens periphery has a similar chemistry. This communication deals with the isolation and characterization of alpha-crystallin from old lenses. This protein was found to be identical to that obtained from cataractous lenses.

![Fig. 1. Bio-Gel A-1.5m chromatography of soluble proteins from six-week-old whole human lens, 65- to 70-year-old normal human lens periphery, and 65- to 70-year-old cataract lens periphery. The eluting buffer is 0.01 M Tris, pH 7.6, 0.1 M KCl.](image)

**Materials and methods**

Normal human eyes were obtained from the New York Eye Bank within approximately 24 hours of death. Cataractous lenses were obtained as described previously.14 The lenses were immediately removed, inspected for clarity and then stored at -84°C. The periphery, defined as the outer 50 to 60 per cent of the lens, was separated from the nucleus of the lens with a No. 3 cork borer. Peripheries or whole lenses were homogenized in 0.01 M Tris, pH 7.6, 0.1 M KCl, and centrifuged at 27,000 g for 15 minutes. The supernatants were fractionated on a 1.5 x 150 cm. Bio-Gel A-1.5m column utilizing 0.01 M Tris, pH 7.6, 0.1M KCl. Pooled fractions were dialyzed...
Results

In order to evaluate some of the age-dependent changes in alpha-crystallin in normal human lenses, the protein was isolated from six-week-old lenses and 65- to 70-year-old lens periphery. The protein was also isolated from 65- to 70-year-old cataractous lens periphery for comparative purposes. After homogenizing the lenses, the HMW and the insoluble fractions were removed and the supernatants from the three different preparations were then passed through a Bio-Gel A-1.5m column. The profiles obtained with the three supernatants are shown in Fig. 1. Based on previous work, alpha-crystallin would be expected to appear in the first peak. It will be demonstrated that this presumption is valid in these experiments. The first peak was eluted somewhat later with the six-week-old lens supernatant than in the preparations of the older lenses appearing in the region anticipated for a macromolecular population of approximately $9 \times 10^5$ daltons. In the old lens preparations, a corresponding peak was observed as well as a component which appeared in the void volume (40 ml.) indicating protein with a molecular weight greater than $1.5 \times 10^6$. This fraction appeared in considerably greater concentration in the preparation from old cataractous lenses than from normal tissue. Earlier work has shown that this fraction contains predominantly alpha-crystallin aggregates greater than $5 \times 10^6$ daltons.

Investigation of the gel electrophoresis profiles in 6M urea and SDS as well as amino acid analyses indicates that the first two peaks from both the old normal and cataractous lenses have identical composition. Thus the presumptive alpha-crystallin is present in different sized populations, the higher molecular weight species being absent in young lens. Because of the limited supply of normal human lenses, the two peaks isolated from old normal lens periphery were pooled for further characterization and fractionation of polypeptide chains.
The amino acid composition of these preparations is shown in Table I. The old normal and cataractous proteins are essentially identical and quite similar to the six-week-old material where significant differences are found in only a few amino acids such as phenylalanine, alanine, glycine, and glutamic acid.

SDS gel electrophoresis of the material obtained from the three preparations is shown in Fig. 2. The material from the six-week-old lenses (gel No. 1) contains only two bands corresponding to molecular weights of 22,000 and 20,000. The older preparations (normal gel No. 2, cataract gel No. 3) also contain significant amounts of a component of approximately 17,000 daltons as well as minor species of higher and lower molecular weight. The pattern obtained from the young lenses is similar to that of calf alpha-crystallin while some of the other bands found in the older preparations have been observed in HMW bovine alpha-crystallins.

The alkaline urea gel patterns of the three preparations and bovine alpha-crystallin are shown in Fig. 3. The nomenclature used for defining the human alpha-crystallin is based upon recent work, with the protein isolated from old cataractous lens periphery where eleven polypeptide chains were observed (Fig. 3, No. 3). An identical pattern was observed with the material isolated from old normal lens periphery (Fig. 3, No. 2). The material isolated from young human lenses contains three major components corresponding to B1, A1, and A2 and minor bands corresponding to B2, B3, B4, and A5 (Fig. 3, No. 1). No evidence of other polypeptides was observed.

Electrophoresis in urea of bovine alpha-crystallin reveals a pattern that is significantly different from that of the human, with bovine B2 corresponding to human B1, bovine B1 to human B3, and bovine A2 to human A1. Bovine A1 does not appear to have a mobility corresponding to any human A chain migrating between human A1 and A3.

To further establish that the normal and cataractous preparations are the same, some of the polypeptides were isolated by DEAE chromatography in the presence of 6 M urea. Profiles similar to those previously reported for alpha-crystallin isolated from cataractous lenses were obtained. Because of the limited amount of normal human material available, only sufficient quantities of B1, B2, B3, and A1 and A2 could be obtained from lenses of older individuals for further investigation. Their amino acid compositions correspond to their counterparts isolated from cataractous lenses. Two dimensional fractionation of the tryptic digests of these polypeptides indicate no differences between normal and cataractous material. Fig. 4 illustrates the patterns obtained with human B1 and B2 and A1 and A2. B1 and B2 each contain one peptide not found in the other. B2 was found to contain two peptides not found in either B1 or B3. A1 and A2 are also al-
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Fig. 4. Fingerprinting of tryptic digests of human B₁, B₂, A₁, and A₂. The designation -B₁ indicates that particular peptide is absent in B₁.

most identical with A₁, containing two peptides not found in A₂ and A₂, one peptide not found in A₁.

Discussion

Previous work with alpha-crystallin isolated from the peripheries of cataractous lenses has unequivocally demonstrated that of the eleven polypeptides found in this macromolecule, four are B chains and six are A chains. One polypeptide designated B₁ requires further investigation to confirm its assignment. The identification of these polypeptides was based upon the isolation and characterization of each chain.

Unfortunately, considerable material is required for such investigation and only a limited number of normal lenses was available for the present investigation. Nevertheless, five polypeptides B₁, B₂, B₃, A₁, and A₂ were isolated in sufficient quantity from normal lens periphery for characterization and comparative studies with the protein from cataractous lenses. Besides the most identical amino acid compositions and SDS and urea gel patterns of all the alpha-crystallins, the polypeptides isolated from normal tissue were identical to their cataractous lens counterpart. Thus it can be concluded that alpha-crystallin isolated from old normal and cataractous lens peripheries have identical polypeptide compositions.

Correlation of the observations upon human NS, young and old alpha-crystallin suggests post-translational reactions similar to those found in the bovine lens. NS protein contains only three polypeptides while in six-week-old lenses, seven polypeptides are observed and in older lenses, eleven polypeptides. Observations upon both cataractous and normal lens alpha-crystallin indicate that the A and B chains are each a group of closely related polypeptides with only minor changes in their chemistries.

It appears that alpha-crystallin in human lenses undergoes a similar age-dependent size transformation to that found in the bovine lens increasing from approximately $7.5 \times 10^5$ daltons to more than $5 \times 10^5$ daltons. The HMW species (greater than $50 \times 10^5$ daltons) found primarily in the nuclear region also appears to contain alpha-crystallin. However, in contrast to the bovine lens, the human HMW fraction is composed of other proteins as well as degraded polypeptides with a molecular weight of approximately 11,000. The so-called insoluble fraction appears to be very similar to the HMW material suggesting that the HMW species may be precursors to the insoluble protein.
tions on the proteins of the nuclear region will be reported in later communications.

The competent technical assistance of Ms. Gloria Jenkins and Ms. Kathy Huang are gratefully acknowledged.

REFERENCES