Studies on lens proteins

III. Variations in polypeptides of lens β-crystallins

M. K. Mostafapour and V. N. Reddy

Relative amounts of two water-soluble β-crystallin polypeptides with molecular weights of 26,000 (26K) and 28,000 daltons (28K) were measured in a number of species and also in different age groups of rats. The data indicate an age-dependent increase in the quantity of the 26K which is concomitant with a decrease in the 28K polypeptide. The ratio of these two polypeptides in the total water-soluble fraction is similar to that observed in the purified β-crystallins. Purified β-crystallin of the rabbit and its equivalent "β1" of the bovine lens display charge heterogeneity upon DEAE chromatography, suggesting subtle qualitative changes in these crystallins. The presence of these two polypeptides in the urea-soluble fraction of lens preparations is also an indication of qualitative changes. The 26K polypeptide of β-crystallins studied here is different from the 26K main intrinsic membrane protein in that, unlike the latter, it does not aggregate upon heating in SDS-buffer.

Key words: mammalian lens, age-dependent variations, 26K and 28K polypeptides, charge heterogeneity, chromatography/electrophoresis/densitometry

Beta-crystallins are a large and diverse group of lens crystallins. Comparative studies of Zigler and Sidbury have revealed the common occurrence of a number of polypeptides in the β-crystallins of various mammalian and submammalian species. Among these common polypeptides are two with apparent molecular weights of 26,000 (26K) and 28,000 (28K). The 26K polypeptide appears to be the same as the polypeptide designated "βBp" by Herbrink and Bloemendal. The latter investigators reported the βBp to have a molecular weight of about 27,000 daltons and to be the major polypeptide common to both the "βH" and the "βL"-crystallins. We have recently shown that the water-soluble fraction of the rabbit lens contains four β-crystallins and that the above two polypeptides are subunits of β3-crystallin. Occurrences of these two polypeptides together as subunits of a specific β-crystallin common to many species can be utilized to study species and/or age-related variations of the lens proteins.

In this communication we report age-dependent quantitative differences in the relative amounts of the 26K and 28K polypeptides of water-soluble β3-crystallin. Chromatographic evidence indicating charge heterogeneity of proteins containing these polypeptides will also be presented.

Materials and methods

Fresh animal lenses were used. Human lenses had been stored at -80°C. Unless otherwise spec-

Fig. 1. Polypeptides of rabbit, human, and bovine lens water-soluble proteins separated by SDS-PAGE. Samples were treated with SDS-mercaptoethanol and electrophoresed on a 5% to 20% acrylamide gradient slab gel with a 3.3% stacking gel. Human and bovine samples are overloaded to show the presence of the 28K polypeptide.

Table I. Variations in the relative amounts of the 26K and 28K polypeptides of β-crystallin

<table>
<thead>
<tr>
<th>Species</th>
<th>A* (26K)</th>
<th>B* (28K)</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>15.3</td>
<td>20.6</td>
<td>0.74</td>
</tr>
<tr>
<td>Frog</td>
<td>18.0</td>
<td>18.6</td>
<td>0.96</td>
</tr>
<tr>
<td>Human</td>
<td>19.3</td>
<td>9.8</td>
<td>1.96</td>
</tr>
<tr>
<td>Bovine</td>
<td>24.6</td>
<td>11.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Cat</td>
<td>18.8</td>
<td>8.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Rat</td>
<td>22.3</td>
<td>9.6</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Water-soluble crystallins obtained from the lenses of species listed were subjected to SDS-PAGE. The relative amounts of each polypeptide were determined by densitometry of the Coomassie blue-stained bands. The total area under all tracing peaks was taken as 100%. The number given in columns A and B represent percent abundances of the 26K and 28K polypeptides. The last column, A/B, represents the changing ratios of these two polypeptides.

Results

Polypeptides of the water-soluble proteins of rabbit, human, and bovine lens preparations separated by SDS-PAGE are shown in Fig. 1. The positions of 28K and 26K polypeptides are shown by arrows. This figure demonstrates that the amounts of these two polypeptides relative to each other were visibly different in each species. Although the 28K polypeptide was predominant in the rabbit, the situation was reversed in the bovine and the human lens where the 26K polypeptide was in excess. Similar comparative studies were carried out on lenses from a number of other animals, and the data are shown in Table I. Values given in columns A and B of Table I indicate that the combined amounts of the 26K and the 28K polypeptides under conditions previously described. The desired peaks were concentrated by freeze-evaporation and rechromatographed on the same column yielding a symmetrical elution peak. Diethylaminoethyl (DEAE)-anion exchange chromatography was performed with Whatman's DE-52 packed in a 2.6 by 40 cm column. The column was equilibrated and washed before and after loading with 0.05M Tris HCl, pH 7.4. Proteins were eluted with a 0 to 0.4M linear NaCl gradient in the same buffer.

The pellet obtained after centrifugation of the first aqueous extract was "washed" free of watersoluble materials by rehomogenization and centrifugation. After three such washes the water-insoluble pellet was stirred overnight at room temperature with TEND buffer containing 8M urea. Samples were diluted to 2M urea and then centrifuged at 100,000 x g for 3 hr. The clear supernatant designated area-soluble fraction was precipitated with 10% trichloracetic acid (TCA). The resulting precipitate collected by low-speed centrifugation was washed once with absolute ethanol to remove excess TCA, and the pellet was dissolved in sodium dodecyl sulfate (SDS)-buffer. SDS-polyacrylamide gel electrophoresis (PAGE) was performed as previously described. Samples were heated for 3 to 4 min in a boiling water bath prior to loading on acrylamide gels. This procedure removes any contaminating 26K intrinsic membrane protein which may be present. Den-
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Fig. 2. Age-dependent changes in rat lens \( \beta \)-crystallin polypeptides. Lenses obtained from animals of different ages were homogenized and subjected to SDS-PAGE as described in Materials and methods. 1, Fetal rat lens, 18 days of gestation (overloaded to show trace amount of the 26K polypeptide); 2, neonatal (1-week-old); 3, 2-week-old; 4, mature.

Fig. 3. Kinetics of age-dependent changes in rat lens \( \beta \)-crystallin polypeptides. Amount of each \( \beta \)-crystallin polypeptide relative to total crystallins was determined by densitometry of the SDS-PAGE patterns. The numbers placed on curves denote molecular weight \( \times 10^3 \).

remained roughly the same (\( \sim 30\% \pm 6 \)). But as shown in column C, the ratio of these two polypeptides to each other changed, and such a change seemed to be due to an increase in the 26K concomitant with a decrease in the 28K polypeptide. Although the data in Table I indicate species differences in the ratios of 26K and 28K, it could not be decided if such changes were either age- or species-related.

In order to determine whether such shifts in the ratios of these two polypeptides are age-dependent, we compared the total lens polypeptide patterns of fetal, neonatal, young, and mature rat lenses. SDS-PAGE results of such an experiment are shown in Fig. 2. The polypeptide bands of interest are the 26K and 28K and also the 30K band seen in lanes 1 to 3 just above the 28K band. These results demonstrate that in the fetal rat the 25K was predominant but that there was a gradual in-
crease in the 26K with age (and a concomitant decrease in the 28K). Dynamics of such changes determined by densitometry, are shown in Fig. 3. An interesting aspect of these data is the transient rise and fall of the 30K band; its quantity increased in the newborn and the young, but decreased thereafter. At present the significance of these changes is not understood, and we do not know whether or not this polypeptide is degraded to lower-molecular-weight polypeptides such as the 26K.

Because the 26K and 28K are subunits of rabbit β\(_{3}\) and bovine \(\beta_{c}\)-crystallins, it is expected that the characteristic ratios of the two polypeptides determined in the total water-soluble fractions remain the same in purified proteins. To investigate this possibility, rabbit \(\beta_{3}\) and bovine \(\beta_{c}\)-crystallins were purified from lens cortex water-soluble fraction by chromatography on Sephacryl-S-200. Fig. 4 shows the chromatographic profiles of rabbit and bovine crystallins. The peak designated \(\beta_{3}\) and shown by an arrow was not present in the bovine preparation. This protein, which has a molecular weight of 130,000 and is composed of one type of subunit with an apparent molecular weight of 33,000,\(^{5}\) seems to be species-specific. Tubes containing the rabbit \(\beta_{3}\)-crystallin and its chromatographic equivalent from the bovine lens were pooled separately, concentrated, and rechromatographed, so that a single symmetrical gel filtration peak denoting homogeneity in terms of molecular weight was obtained. Each peak was pooled, dialyzed against 0.05M Tris HCl, pH 7.4, and adsorbed onto a DEAE column. The column was washed and finally eluted with a 0.0M to 0.4M NaCl gradient. The elution profiles of rabbit and bovine proteins eluted from the DEAE column are shown in Fig. 5. That the homogeneous peak from gel filtration chromatography was resolved into several peaks in each case signified charge heterogeneity within each purified protein. SDS-PAGE patterns of DEAE fractions of rabbit \(\beta_{3}\)- and bovine \(\beta_{c}\)-crystallin are shown in Fig. 6. It can be seen that all the rabbit \(\beta_{3}\) subfractions (Fig. 6, A) contained a higher amount of the 28K but that all bovine subfractions displayed a larger quantity of the 26K polypeptide (Fig. 6, B). In both cases, the relative quantities of these two polypeptides were similar to those found in the total water-soluble fractions (Fig. 1).

Roy and Spector\(^{8}\) have previously reported that the water-insoluble, but urea-soluble high-molecular-weight aggregates of the lens originate from the water-soluble crystallins. It was of interest therefore to know whether the above subunits were present in the urea-soluble fractions because this would indicate

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Fig. 5. Charge heterogeneity of rabbit \(\beta_{3}\)- and bovine \(\beta_{c}\)-crystallins. The \(\beta\)-crystallins purified to homogeneous molecular weights by gel filtration (see Results) were adsorbed onto a DEAE column and eluted with a 0.0 to 0.4M Tris, pH 7.4. Broken line, Rabbit profile; solid line, bovine.
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Fig. 6. Comparison of the rabbit β3- and bovine βL-crystallin subfractions. Protein peaks eluted from the DEAE column shown in Fig. 5, were subjected to SDS-PAGE. A, Rabbit lens. M, Marker proteins (mol. wt. × 10^-6); lanes 1 to 3, polypeptides of β3-subfractions. Note that all fractions have a similar pattern, with the 28K polypeptide being in excess. B, Bovine lens. Lanes 1 to 4, βL-subfractions (note excess amount of the 26K polypeptide in all subfractions); lane 7, total water-soluble protein polypeptides of the bovine lens.

that further modifications had taken place. In confirmation of their finding we observed the presence of the 26K and the 28K polypeptides in our urea-soluble fraction (data not shown). The 26K polypeptide of the urea-soluble fraction did not aggregate upon heating in SDS-buffer, and by this criterion it is different from the 26K main intrinsic proteins which aggregate upon heating and do not enter the gel, as originally shown by Wong et al.7

Discussion

We have presented data on the distribution and relative abundance of two polypeptides (26K and 28K) of the lens β-crystallins in a number of species. Although there are variable quantities of these two polypeptides in the species studied, we have demonstrated that these differences are age-dependent. To the extent that the species studied were of different ages, the ratios of the polypeptides measured may not be characteristic of the species per se. In a previous study Zigler9 reported changes in the percent abundances of the proteins and polypeptides of fetal calf, calf, and bovine lenses, but no consistent trends of either an increase or a decrease in the quantity of polypeptides were discernible. In our study, using laboratory rats, we have monitored dynamics of age-related changes in the two specific β-crystallin subunits. The data show clear age-dependent shifts in the abundance of these polypeptides (Fig. 3). There seems to be a trend toward accumulation of the 26K polypeptide with age which is concomitant with a decrease in the quantity of the 28K (and also 30K). The reasons why such shifts occur are open to speculation. Some parallels can be found between these in vivo shifts and those observed in vitro in the chick lens. Shinohara and Piatigorsky10 have shown that changes in the ionic ratios of Na+ and K+ do effect a shift in the rate of synthesis of chick β-crystallin subunits. We do not know whether similar or more subtle ionic shifts in the developing rat eye are the causative factors for the differential synthesis of lens polypeptides. The possibility that accumulation of the 26K polypeptide is a result of degradation of higher-molecular-weight polypeptides such as the 28K and 30K species needs to be investigated.

Because the 26K and 28K polypeptides occur together as subunits of β3-crystallins, their changing ratios are an indication that they can assemble in different proportions to make up the β-crystallin molecule in a manner analogous to isozymic structures. That the β3-crystallin of the rabbit and its equivalent βL-crystallin of the bovine lens have similar molecular weights (Fig. 4) but have different ratios of constituent polypeptide subunits (Fig. 5) lends support to this idea.

Our previous data on cross-sectional SDS-PAGE profiles of human lenses indicate...
an age-dependent loss of higher molecular weight and an increase in the 10,000 to 20,000 range polypeptides. Age not only affects the quantity of specific lens polypeptides but also can cause subtle qualitative changes as well. The charge heterogeneity observed in the ββ-crystallin of the rabbit and the βL-crystallin of the bovine lens upon DEAE column chromatography (Fig. 5) is evidently the result of charge differences in their constituent polypeptides. Our DEAE chromatography was performed under non-dissociating conditions; therefore we cannot tell if the charge heterogeneities are due to one or the other of the polypeptides. It is possible that subtle qualitative changes which occur by age may be involved in the increased production of lens water-insoluble proteins.

In summary, our results indicate age-dependent changes in specific lens polypeptides. Such changes must be considered when comparing lens proteins of different species of varying ages. Further studies of age-related variations in lens proteins, especially of the human lens, could lead to a better understanding of processes which lead to senile cataract.

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REFERENCES