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Racemization in human lens: Evidence of rapid insolubilization of specific polypeptides in cataract formation*

(D-aspartic acid/soluble and insoluble protein/43,000- and 10,000-dalton polypeptides)

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ABSTRACT **After early life, the dry weight of normal human lenses increases at a relatively constant rate with time. Transformation from soluble to insoluble material appears to occur at a comparable rate, resulting in a constant amount of soluble material. However, in cataract the insolubilization rate is accelerated. These observations are supported by determination of D-aspartic acid/L-aspartic acid ratios. The abundance of D-aspartic acid increases with aging at a constant rate in the insoluble fraction of normal lenses but does not change in the soluble fraction. However, in cataractous lenses there is a significant decrease in the ratio in the insoluble fraction. Examination of polypeptides isolated from reduced and alkylated soluble and insoluble cataractous lens protein as well as other data suggest the following additional conclusions: (*i*) the 10,000-dalton polypeptide in the insoluble fraction is derived in part from degradation of an already insoluble precursor; and (*ii*) the lowered abundance of D-aspartic acid in the insoluble fraction of cataractous lenses is primarily due to the rapid insolubilization of the 43,000- and 20,000-dalton range components.**

It was shown by Hare and Abelson (1) and then by others (2–5) that the abundance of racemized amino acids (particularly aspartic acid) is a measure of the aging of biological material (6). Such a parameter may be used to examine precursor relationships in the development of cataract. Recently, Masters *et al.* (5) have demonstrated that racemization of aspartic acid occurs at a constant rate of 0.14% per year in the inner region of both normal and cataractous lens. However, such experiments give no insight into precursor relationships between lens proteins.

The lens is composed primarily of protein which comprises most of the dry weight (7). It has been previously shown that the relative percentage of water-insoluble material increases with aging in both animal (8) and human (9–11) lenses and in the development of lens opacities (9–11). It was therefore of interest to compare the relative rates of insolubilization of material from normal and cataractous lenses. If the rate of insolubilization in cataract is increased, it might be expected that the abundance of D-aspartic acid would be decreased in the resulting insoluble fraction. This would be the case if the soluble lens protein representing a younger population of material was more rapidly converted to the insoluble fraction. The present report demonstrates the validity of these assumptions.

EXPERIMENTAL

All human lens were freshly obtained and stored at −80° prior to use. Lenses were classified as normal or cataractous as described by Anderson and Spector (12). Cataracts of type 4 in color and type 4 in opacity were utilized. The term "water-soluble fraction" describes the 60,000 × *g* supernatant obtained after homogenization of individual normal and cataractous lens in 0.1 M KCl/0.05 M Tris, pH 7.4. The water-insoluble fraction represents the pelleted material obtained from the above procedure and contains, in addition, the so-called high molecular weight protein fraction. The water-soluble fraction was dialyzed against deionized water at 4° for 48 hr. The water-soluble and -insoluble fractions were transferred to previously weighed vials and lyophilized for 72 hr.

Aspartic acid was isolated from acid hydrolysates of 4- to 10-mg samples. Hydrolysis was performed in constant-boiling HCl for 22 hr in evacuated, sealed tubes. The samples were dried and the aspartic acid was localized after elution (13) from a Hamilton AN-90 column (Pierce Chemical Company) by inclusion of 2.5 pmol of L-[2,3-^{3}H(N)]aspartic acid (New England Nuclear; 15.0 Ci/mmol). The D/L ratio of D-aspartic acid to L-aspartic acid (D/L Asp) was determined by the diastereomeric dipeptide method (14).

The subunit polypeptides of 65- to 72- year-old cataractous lens protein were isolated from Sephadex G-150 and G-100 equilibrated with 10% HOAc and 7.2 M urea as described elsewhere (15). All polypeptides described were reduced and alkylated prior to their purification unless otherwise noted.

RESULTS AND DISCUSSION

When the total dry weight (water-soluble plus water-insoluble) of normal lenses is plotted against age, after the first few years of rapid growth an increase in apparent weight of approximately 0.4 mg/yr is found (Fig. 1). Similar results have been observed by Klethi (17). Determination of the dry weight of the water-insoluble fraction suggests that it increases at the same rate as the total dry weight. Such observations indicate that the absolute amount of the water-soluble fraction remains constant at approximately 30 mg, and the rate of conversion of soluble to insoluble material is constant in the normal lens. Thus, the percentage of insoluble material increases at a constant rate from about 2% at birth to approximately 50% by about age 70 years.

When individual cataractous lenses were examined (open circles in Fig. 1), the total weight generally corresponded (within experimental error) to that of normal lenses of the same age group. However, the amount of the water-insoluble fraction in the cataractous lenses (solid circles) was dramatically greater than in the normal lenses, suggesting a more rapid conversion of soluble to insoluble material. Thus, it would appear that a relatively rapid loss of water-soluble protein may be correlated with lens opacity.

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Abbreviation: D/L Asp ratio, ratio of D-aspartic acid to L-aspartic acid.

* This investigation was initially reported at the Cataract Cooperative Research Group meeting on Apr. 3, 1978, in Washington, DC.

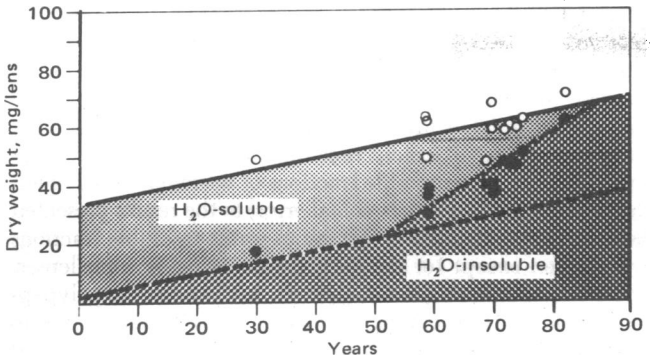

FIG. 1. Comparison of growth of cataractous (○, ●) and normal (—, ---) lenses shown as a plot of dry weight versus age for the total water-soluble plus water-insoluble fractions (—, ○) and the water-insoluble fractions (---, ●) as determined from human lenses. The data for the normal lenses are based on $n = 19$ with a linear correlation coefficient >0.95 for both the total lens dry weight (SD ±3.7) and the water-insoluble fraction (SD ±1.9) (16). Superimposed upon these lines are the respective values obtained from individual cataractous lenses. The apparent rate of new insolubilization for cataractous lenses is shown as a dotted line. The water-insoluble fraction is denoted by the large-dot stippling.

Examination of the degree of aspartic acid racemization found in the water-soluble and water-insoluble fractions of normal lens (Fig. 2) indicated that the water-soluble fraction D/L Asp ratio of 0.036 remains quite constant with age whereas the ratio in the water-insoluble fraction increases at 0.17%/yr.

Such results imply that it is the oldest soluble lens proteins that are converted to the insoluble fraction, thus maintaining a soluble protein fraction with a relatively low abundance of D-aspartic acid independent of age. The linear increase in accumulation of D-aspartic acid in the insoluble fraction reflects the apparent consistency in the rates of racemization in the soluble and insoluble fractions coupled with the constant rate of insolubilization. These results also suggest the possibility that, in the normal aging process, racemization of L-aspartic acid may contribute to perturbation of protein structure which may result in aggregation and insolubilization.

Masters *et al.* (5) have shown no apparent change in the overall rate of racemization in the nuclear region between cataractous and normal lenses. Therefore, the water-insoluble fraction of cataractous lenses should contain a lower D/L Asp ratio than corresponding normal lenses of the same age because there is a more rapid transformation to the water-insoluble component in cataractous lenses (Fig. 1). A comparison of D/L Asp ratios from individual cataractous lenses with D/L Asp ratios for normal lenses appears to confirm this hypothesis (Table 1). In almost every case, the observed D/L Asp ratio from the water-insoluble fraction of cataractous lenses was less than the D/L Asp ratio expected for a corresponding normal lens.

In order to examine this situation more closely, the polypeptides comprising the lens proteins were isolated by recently developed purification methods (15). Both the soluble and insoluble fractions of older normal and cataractous lenses contained major polypeptides with molecular weights of 10,000, ~20,000, 43,000, and 60,000. In addition, the insoluble fraction also contained a component >100,000 daltons that appeared in some fractionation systems. The latter fraction isolated from cataract differed markedly in chemical and physical properties as well as in abundance from its normal lens counterpart (18).

The amount of racemization of aspartic acid was determined for each of these subunits isolated from cataractous lenses (ages 65–72 yr) as shown by the bars in Fig. 2. A minor 80,000-dalton

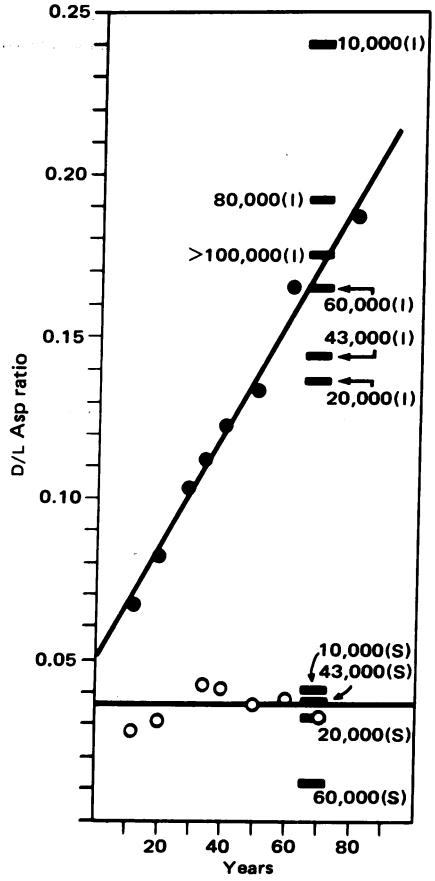

FIG. 2. Comparison of the rate of racemization of aspartic acid found in the total water-insoluble (●) and total water-soluble (○) fraction of normal human lenses. The various size polypeptides isolated from the water-soluble fraction (S) and water-insoluble fraction (I) of cataractous lenses between the ages of 65 and 72 yr are denoted by the bars.

fraction was also isolated from the insoluble fraction. In all cases the material was reduced and carboxymethylated before isolation. The D/L Asp ratios of the soluble polypeptides were either approximately the same as the ratios of total normal soluble protein or, as in the case of the 60,000-dalton component, less than that. Such results suggest that the polypeptides

Table 1. Comparison of D/L Asp ratios of the water-insoluble fractions of cataractous and normal lenses

Age, yr	Type*	D/L Asp ratio	
		Cataract	Normal†
30	C	0.105	0.101
59	NC	0.099	0.151
59	C	0.098	0.151
59	C	0.124	0.151
69	NC	0.123	0.168
70	C	0.116	0.170
70	C	0.130	0.170
71	N	0.163	0.171
72	NC	0.107	0.173
73	N(Y)	0.175	0.175
74	NC	0.109	0.177
75	N(Y)	0.161	0.178
82	N(Y)	0.163	0.190
82	N(Y)	0.171	0.190

* All cataracts were type +4 (12). Y, Yellow nucleus; N, nuclear involvement; C, cortical involvement.
† Based on data from Fig. 1.

from the water-soluble fraction all have been recently added to the soluble pool to proteins, either by direct synthesis or by relatively rapid post-translational degradation. However, the results obtained with the individual polypeptides from the water-insoluble fraction of cataractous lenses had a wide range of D/L Asp ratios, suggesting a number of independent mechanisms by which the polypeptides may arise in this fraction.

It now appears that the 10,000-dalton polypeptide arises from degradation of the 20,000-dalton α-crystallin A-chain (19). This conclusion is based upon isolation and characterization of peptides isolated from tryptic digests of the 10,000-dalton polypeptide. Excellent correspondence in residues 12–70 was found between the reported primary sequence of the human α-crystallin A-chain (20) and the 10,000-dalton component. In the soluble fraction, the 10,000-dalton component remained relatively constant (>30 yr; 7%) with aging, whereas there was a continual decrease (0.29%/yr) in the 20,000-dalton soluble component (16). However, the decrease in the 20,000-dalton soluble component was not sufficient to explain the dramatic age-dependent increase (>30 yr; 0.58%/yr) in the insoluble 10,000-dalton polypeptide. The marked age-dependent decrease (0.29%/yr) in the insoluble 20,000-dalton component suggests that the observed increase in the insoluble 10,000-dalton polypeptide is, to an appreciable extent, due to the degradation of the insoluble 20,000-dalton species. Such a mode of formation could be expected to increase the D/L Asp ratio of the insoluble 10,000-dalton fraction. As shown in Fig. 2, the D/L Asp ratio of the insoluble 10,000-dalton component was markedly higher than the average ratio for normal insoluble protein. Because the 10,000-dalton polypeptide from the soluble fraction has been shown to be closely related to its insoluble counterpart, it is unlikely that the structure of the insoluble polypeptide would induce a different rate of racemization.

All of the ≥60,000-dalton polypeptides had D/L Asp ratios in the range of the ratio of the water-insoluble fraction of normal lens. Therefore, these polypeptides are probably entering the insoluble fraction at a rate similar to that found in normal lenses. However, the 43,000- and ~20,000-dalton polypeptides had D/L Asp ratios that apparently were decreased by accelerated entry of newly synthesized subunits into the insoluble fraction. It has been shown (18) that, in cataract, a disulfide-linked high molecular weight aggregate is formed which contains the ~20,000- and 43,000-dalton polypeptides. This disulfide-linked aggregate is only found in the insoluble fraction. It probably contributes significantly to the increased rate of insolubilization in cataract and probably represents a large proportion of the population of 20,000- and 43,000-dalton components isolated from the insoluble fraction after reduction. Cataractous insoluble high molecular weight protein contains these components as well as a nondescript entity that is also found in normal lenses and behaves as an apparently >100,000-dalton component after reduction. When the high molecular weight fraction was isolated before and after reduction and alkylation, the D/L Asp ratios were 0.156 and 0.174, respectively. This again reflects the recent addition of 20,000- and 43,000-dalton polypeptides to the water-insoluble fraction.

Because no increased degree of overall racemization is found in senile cataractous lenses (5), other processes must be involved in the observed accelerated insolubilization. The primary cause may be oxidation of the soluble protein (8, 18) to produce insoluble covalently linked aggregates. It has been suggested that the 43,000-dalton polypeptide is an extrinsic membrane protein (ref. 16; M. K. Mostafapour and V. N. Reddy, personal communication). Because, from the results presented here and elsewhere (18), the 43,000-dalton polypeptide has been implicated with the cataractous process, it is possible that this polypeptide may represent a nucleation site for interaction between the soluble proteins and the membrane or cytomatrix.

Since this manuscript was completed, Masters *et al.* (21) published a related investigation. Similar comparative measurements of aspartic acid racemization between the water-soluble and water-insoluble fractions of normal lenses yielded data similar (within experimental error) to the results presented here. No data on human cataractous water-insoluble fractions were given except for the special case of brunescent lenses. Some variation was noted in the D/L Asp ratio in the polypeptides isolated from cataractous lenses, which may be due to differences in isolation procedures. Unfortunately, Masters *et al.* did not report the D/L Asp ratios for the individual polypeptides isolated from the water-soluble fraction. They concluded that fractions that are crosslinked or degraded have the highest D/L Asp ratios, thus implying that racemization may be a requirement for further degradation or insolubilization. However, our observations indicate that (*i*) the 10,000-dalton polypeptide in the soluble fraction (apparent degradation product of the 20,000-dalton A-chain) occurs without any relative change in the abundance of D-aspartic acid; and (*ii*) the 43,000- and ~20,000-dalton components in the water-insoluble fraction have lower D/L Asp ratios because of rapid insolubilization of the respective soluble species. This latter process results in the lowered D/L Asp ratios observed for the overall polypeptide populations measured in the total water-insoluble fraction of human cataractous lenses.

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