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PRESENCE OF LOW MOLECULAR WEIGHT POLYPEPTIDES IN HUMAN BRUNESCENT CATARACTS

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Microdissected sections from opaque & brunescent lens nuclei contain low molecular weight (4,000-8,000 dalton) polypeptides not found in microdissected sections of transparent lens nuclei. Tryptic digestion of these polypeptides from different cataracts reveal similar peptide maps. Together, these results support the involvement of proteolysis in human cataractogenesis, and suggest the possibility of similar molecular mechanisms occuring in cleavage of lens polypeptides during formation of the opaque & brunescent human cataract.

Extensive characterization of proteins from the human lens has suggested significant covalent modifications of these polypeptides during human cataract development (1). One of the changes best documented has been covalent crosslinking of polypeptides to form high molecular aggregates (2-4). Conversely, studies of cataracts from animal model systems (5,6) have suggested that lens polypeptides may also be cleaved into lower molecular weight components during lens opacification.

Nonetheless, compelling evidence for the presence of such protein breakdown products in human cataracts has so far not been reported. Since significant amounts of proteases have been detected in the human lens (7-9), it is highly possible that proteolysis may occur during cataract formation. To test this hypothesis, we have compared the polypeptide composition of microdissected sections from opaque versus transparent parts of the human lens. The results indicate the presence of low molecular polypeptides in opaque & brunescent sections of human cataracts, & strongly suggest the process of lens protein proteolysis during human cataract formation.

Materials and Methods

Sample preparation: Following removal & classification of the lens (10), the nucleus was separated as previously described (11). The nuclei studied in

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this report were of two general types. The first control group were transparent, lightly-pigmented, and were obtained from normal eye bank eyes or from cataracts with opacities in other parts of the lens (cortex or posterior subcapsular region). The second group were obtained from cataracts & were completely opaque & brunescent (brown to dark brown color). After freezing, the nucleus was sliced into 160 micron sections and sections taken from the center of the nucleus were homogenized as previously described (11).

HPLC: The protein content of the supernatant following centrifugation was determined using bovine serum albumin as standard (12), & approximately 2-8 micrograms was analyzed by HPLC using gel permeation chromatography (11). Where appropriate, peak fractions were collected, dialyzed against 1 mM NH_4HCO_3 using a 1,000 dalton cut-off membrane (Spectrapor), & lyophilized.

Isoelectric focusing followed by sodium dodecylsulfate polycrylamide gel electrophoresis (IEF X SDS): Two micrograms of protein used for HPLC analysis was radioiodinated with I^{125} as previously described (13), then resolved in two dimensions essentially according to O'Farrell (14). Following staining & destaining (13), proteins were visualized by autoradiography & excised from the gel.

One-dimensional analysis (SDS-PAGE): Samples were radioiodinated (13), then resolved on 12.5% polyacrylamide gels (15).

Peptide mapping: The individual spots excised from the gel were digested with trypsin & mapped as previously described (13), then exposed to XRP-1 autoradiography film.

Results

Figure 1 demonstrates results of HPLC analysis of soluble proteins from microdissected sections from transparent versus opaque & brunescent nuclei. In both transparent nuclei (Fig. 1a & 1b), various protein peaks eluting between the void volume (11.8 min) & approximately 24.2 min were observed. These profiles are representative of four other transparent nuclei, obtained from normal lenses 35-72 years of age. The most striking difference between transparent versus opaque & brunescent nuclei is the presence in the latter of low molecular weight components eluting at approximately 25 min & later (Fig. 1c). Two such peaks eluting at 25.0 & 26.0 min are seen from a representative microdissected sample from an opaque & brunescent nuclei from the lens of a 78 year old patient. Microdissected samples from 8 other opaque & brunescent nuclei from patients 69-88 years of age also showed the presence of major peaks eluting 25.0 min & later.

Since the HPLC analysis was conducted in the absence of dissociating and/or reducing conditions, IEF x SDS of radioiodinated samples in the presence of 2-mercaptoethanol was used to analyze the same samples seen in Fig. 1. Both microdissected sections from transparent nuclei (Fig. 2a & 2b) contain various polypeptides greater than approximately 12,000 daltons. In

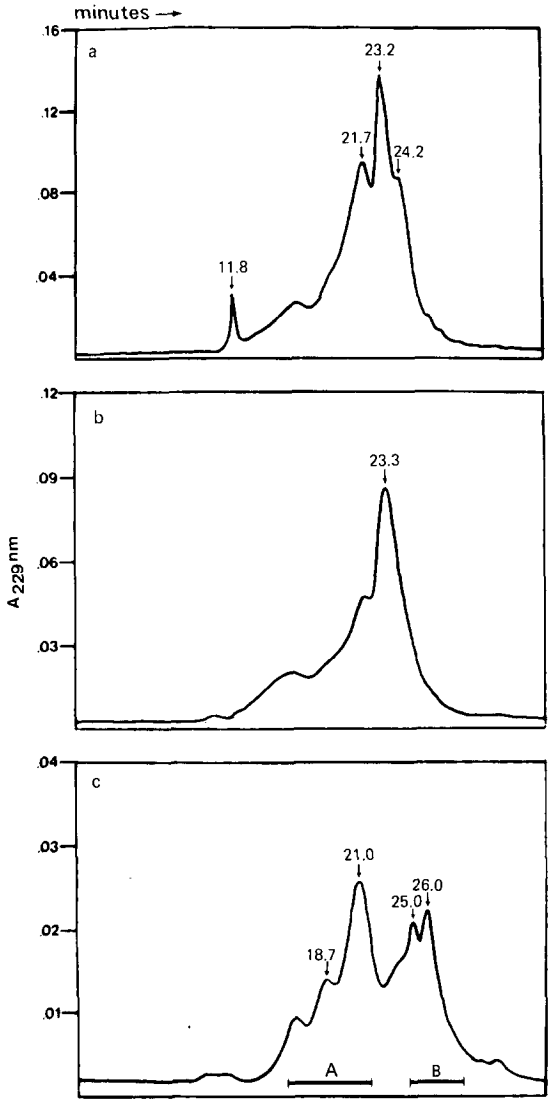

Figure 1: HPLC analysis of soluble proteins from microdissected sections of normal & cataractous nuclei. See Materials & Methods & (11) for further details of analysis. Elution times of major peaks are designated by vertical arrows. a, 53 year old normal donor; b, transparent nucleus from an 82 year old patient with cortical opacification; c, opaque & brunescent nucleus from a 78 year old patient. Horizontal bars in c represent fractions taken for further analysis.

addition, the microdissected section from the opaque & brunescent nucleus (Fig. 2c) contains low molecular weight polypeptides that appear in IEF x SDS patterns as a diffuse spot with a broad molecular range of approximately 4,000-8,000 daltons.

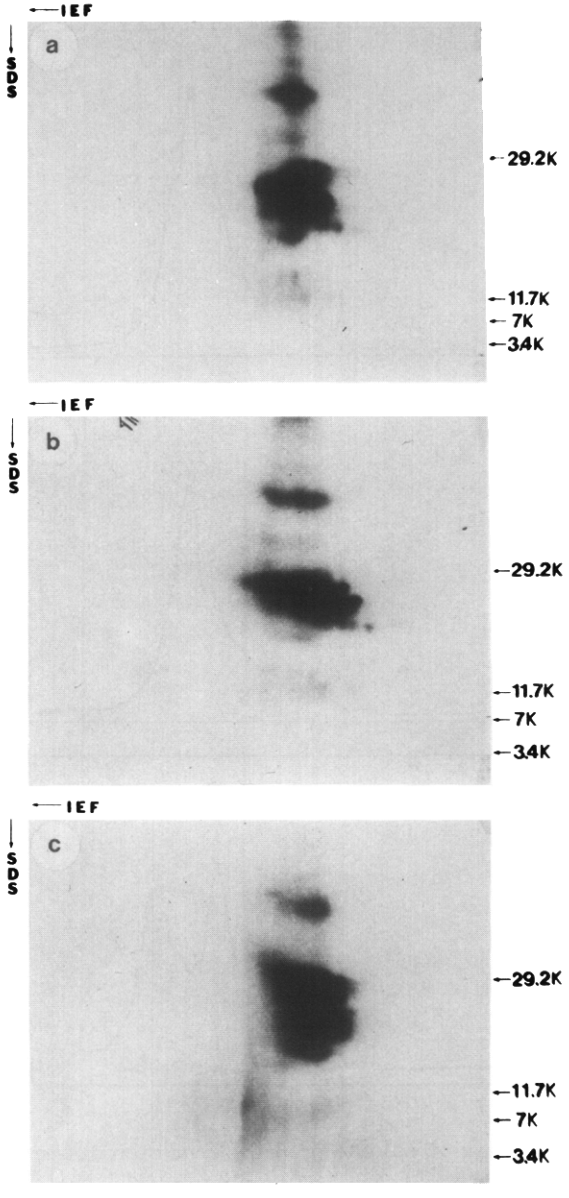

Figure 2: IEF x SDS of radioiodinated samples analyzed by HPLC. Horizontal arrows designate the migration of molecular weight markers. Each panel corresponds to the same samples analyzed by HPLC in Figure 1.

To confirm that the low molecular components seen in Fig. 2c were indeed the same as those observed in Fig. 1c, fractions were collected during HPLC, then dialyzed, lyophilized, radioiodinated, & analyzed by one-dimensional SDS-PAGE. Fig. 3 shows that the 25.0 min & 26.0 min peaks of Fig. 1c are comprised mainly of lower molecular weight components, including the low

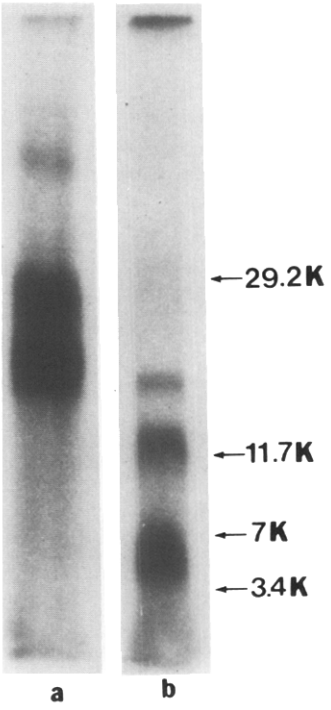

Figure 3: SDS-PAGE of fractions from HPLC. See Fig. 1c for identity of fractions that were radioiodinated, then resolved on 12.5% polyacrylamide gel. Horizontal arrows denote molecular weights of the same marker polypeptides used in Fig. 2. a, higher molecular weight polypeptides (fraction A of Fig. 1c); b, low molecular weight polypeptides (fraction B of Fig. 1c).

molecular weight component of approximately 4,000-8,000 daltons seen in Fig. 2c.

Figure 4 illustrates the tryptic peptide maps of the low molecular weight material from two different opaque & brunescent nuclei. If proteolytic sites were completely random, no unique peptides would be seen. Rather, the peptide map would be quite diffuse. Although there is some diffuse material seen in the peptide maps shown in Fig. 4, most of the material is present in distinct peptides. Furthermore, the peptide maps of the low molecular weight material from these and 7 other opaque & brunescent nuclei were very similar, suggesting that a common proteolytic mechanism was occuring during formation of these human cataracts.

Discussion

The presence of increased amounts of free amino acids (6) & low molecular weight polypeptides (5) have suggested that proteolysis occurs during cataract

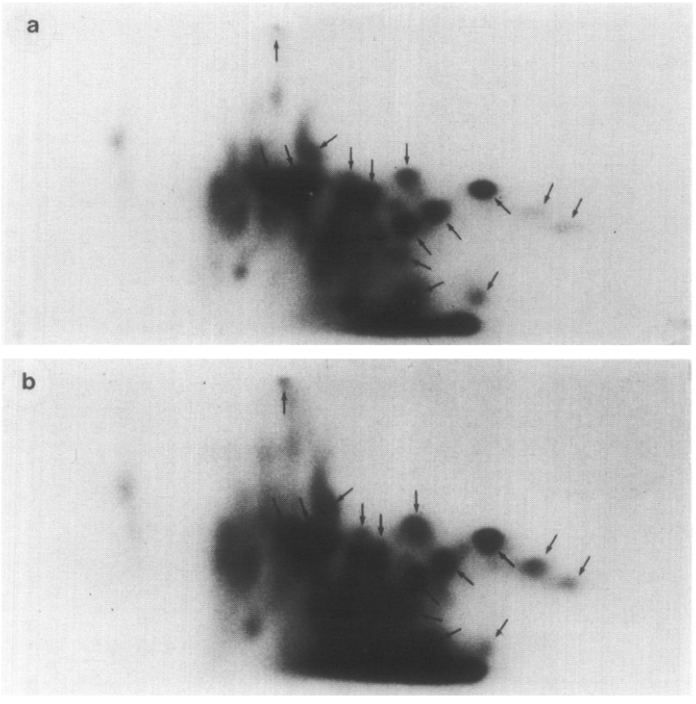

Figure 4: Tryptic peptide maps of radioiodinated peptides from the low molecular polypeptides of opaque & brunescent lens nuclei. The low molecular polypeptides of approximately 4,000-7,000 daltons were excised from the dried IEF x SDS gel, digested with trypsin, & resolved in two-dimensions. Arrows indicate major peptides that were common to both lenses. a, low molecular weight material from lens of an 80 year old cataract patient; b, low molecular weight material from lens of a 78 year old cataract patient.

formation in animal model systems. To date, however, there has been no direct evidence suggesting that proteolysis actually occurs in human cataracts.

Based upon this report, microdissected sections from opaque & brunescent human lens nuclei contain low molecular polypeptides not detectable in transparent nuclei of the same age range. Because of the virtual absence of protein synthesis in the human lens nucleus, the most probable origin of these low molecular polypeptides would be via posttranslational modification of higher molecular weight components during opacification & pigmentation of the human lens nucleus. The molecular nature of this modification could involve nonenzymatic and/or enzymatic modes of cleavage. Possible insult to the human senile lens due to photo-oxidation (3,16) could result in some nonenzymatic cleavage of peptide bonds.

Alternatively, the formation of low molecular weight polypeptides during cataract formation may be the result of endogeneous lens proteases. This latter possibility is consistent with the known presence of human lens proteases active at physiological pH (7,8,17). Self-proteolysis (autolysis) of normal human lens extracts has indeed been shown to occur *in vitro* following the addition of cations (7).

Not only do the results of HPLC & IEF x SDS analyses argue strongly for the presence of *in situ* proteolysis during human cataractogenesis, but peptide mapping of the low molecular polypeptides produced during cataractogenesis also provide clues as to the mechanism of proteolysis. Comparison of peptide maps of low molecular weight components from different lenses demonstrate the presence of distinct peptides. These results indicate that proteolytic cleavage is not random, but must involve the preservation of certain "core" sequences seen as distinct spots. The similarities of these maps argue strongly for the presence of a common mechanism of proteolysis that consistently occurs in human lenses with both nuclear opacification & brunescence.

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