CHEMISTRY OF LENS NUCLEAR SCLEROSIS*

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Two forms of insoluble protein have been isolated from the rat lens based upon their solubility in 7M urea. The urea soluble form, found mainly in the cortex of the lens, contains nearly equal levels of alpha and gamma crystallins, and appears to be associated by hydrogen and hydrophobic bonds. The urea insoluble form, found mainly in the nucleus of the lens contains mostly gamma crystallin, and is held together by covalent linkages, such as SS bonds. The data strongly indicates that nuclear sclerosis during aging is due to the conversion of the urea-soluble form to the urea-insoluble form in the nucleus dependant upon these factors: dehydration, SH unmasking, pressure, and gamma crystallin content.

As a consequence of aging, lenticular elasticity and transparency are reduced. This change in the lens is accompanied by a more rapid increase in the level of the insoluble proteins than of the soluble proteins (Figure 1).

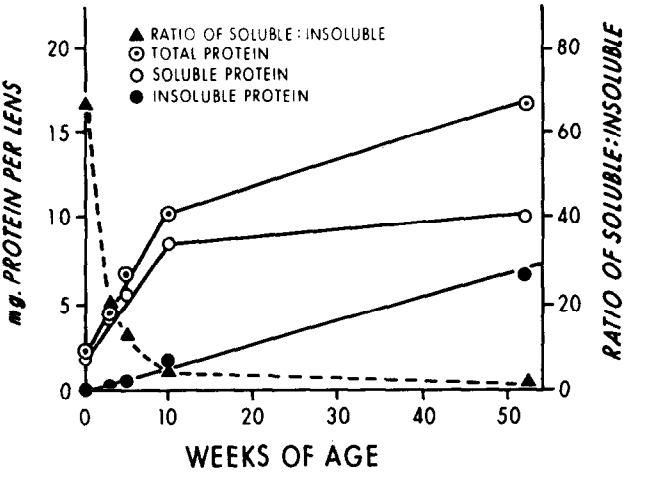

Figure 1. Changes in rat lens protein levels with age. Proteins were estimated by the Lowry method (15).

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Fulhorst and Young (1) have shown that insoluble lens proteins are derived from preexisting soluble proteins, and not from newly synthesized material. Many workers have also reported that alpha crystallin is the soluble protein most closely related to the insoluble fraction of the cow lens (2,3,4), while others have reported that the insoluble fraction of the rat lens closely resembles gamma crystallin (5,6,7). Recent immunochemical studies (8) have shown that the insoluble fraction of both species contains both of these soluble proteins, with alpha predominating in cow lens and gammapredominating in rat lens. Beta crystallin also seemed to be present in both insoluble proteins.

The apparent discrepancies between the composition of cow and rat lens insoluble proteins were at least partly related to differences in preparative procedures. In the cow studies, the 7M urea solubilized portion of the water insoluble fraction was used for analysis. An insoluble residue was left which was not quantitated or characterized. In the rat studies, the whole water insoluble fraction was dissolved by sulfonation of SS and SH bonds which produced a completely water soluble product. In the present work both procedures were combined to obtain two portions of the total water insoluble protein (TIP) on the basis of solubility in 7 M urea.

After homogenization of Holtzmann rat lenses in water and removal of all remaining soluble protein by repeated resuspension and recentrifugation at 2000 rpm at 10°C, the TIP was suspended in 7M urea for ½ hr and the 7M urea soluble (US) portion was removed by centrifugation (100,000 xg for ½ hr). The residue , or 7M urea insoluble portion (UI) was solubilized by treatment with Bailey's reagent (SO_3^-, $S_4O_6^-$, 7M urea, pH 9.9) which sulfonated all SS and SH groups. Addition of mercaptoethanol to the solubilized UI fraction resulted in reformation of an insoluble product. The US fraction therefore represents a noncovalently linked, weakly associated protein aggregate, probably held together by hydrogen bonds and hydrophobic attractions, whereas the UI fraction is firmly held together by strong covalent bonds, such as SS bridges and by others not as yet characterized. Changes in the relative amounts of these two portions of the TIP of rat lenses are shown in

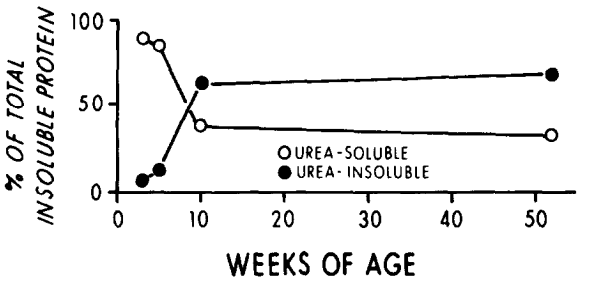

Figure 2. Changes in rat lens insoluble proteins (US and UI) with age. Proteins were estimated by the Lowry method (15).

Figure 2. The most rapid change takes place between 5 and 10 weeks of age, when the predominance of US in lenses of the younger animals is replaced by a predominance of UI in the older animals. Coincidental with this fairly abrupt changeover is the slowdown of gamma crystallin synthesis which results in a drop of gamma crystallin level to below 50% of the total soluble protein. Loss of the ability of the lens to cloud at temperatures below 10°C also occurs precisely at this age (5), a fact which emphasizes the marked decrease in the level of soluble gamma crystallin.

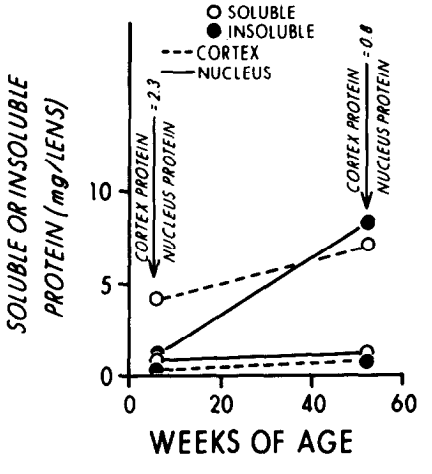

Figure 3. Changes in cortical and nuclear protein levels with age. Proteins were extimated by the Lowry method (15).

During aging of the lens a hard central nucleus forms. As seen in Figure 3 the ratio of total lens cortex protein to total nucleus protein changes from 2.3

in 6 week old rats to 0.8 in 52 week old rats. The figure further illustrates that during this interval the amount of soluble protein in the cortex drops below the amount of insoluble protein in the nucleus. During this time the US drops from 85% of the TIP in the nucleus to only 25% of TIP, concomitant with an increase of UI from 10% to 65% (Figure 4). It seems clear that the increase in size and hardness of the

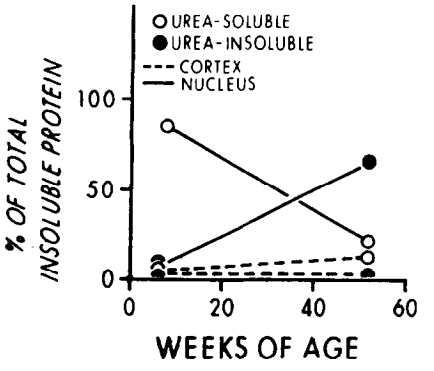

Figure 4. Changes in cortical and nuclear US and UI distribution with age. Proteins were estimated by the Lowry method (15).

Table I

Amino Acid Analyses* of Rat Lens Sulfo-Proteins

Amino Acid	Urea Insoluble (U.I.)	Urea Soluble (U.S.)	Gamma Crystallin	Alpha Crystallin
	Residues of Amino Acid per 1000 Residues			
Lysine	23	31	25	42
Histidine	35	30	35	30
Arginine	119	102	111	73
Aspartic Acid	107	102	106	97
Threonine	27	31	30	35
Serine	80	108	76	107
Glutamic Acid	137	130	127	110
Proline	49	56	48	73
Glycine	89	89	87	74
Alanine	25	38	29	39
Valine	43	50	50	63
Methionine	30	15	30	15
Isoleucine	36	36	36	39
Leucine	72	75	70	87
Tyrosine	81	58	83	38
Phenylalanine	44	49	57	77

*Hydrolysed at 110°C; 24 hrs.; 6N HCL.
Average of two determinations.

lens nucleus closely accompanies the accumulation of urea-insoluble at the expense of urea-soluble protein.

Preliminary identification of the proteins present in the US and UI fractions by amino acid composition analysis (Table I) indicates that UI is nearly 100% gamma crystallin while US is approximately 50% gamma and 50% alpha. A small amount of beta contamination has caused the values for glutamic acid to be higher than those of both alpha and gamma crystallins. When the values of gamma and alpha crystallins in 52 week old rat lenses were determined by ultraviolet absorption analysis (Figure 5), cortical US appears to contain only alpha; nuclear US contains 60% gamma; cortical UI contains 70% gamma; and nuclear UI is 80% gamma crystallin. A similar relationship of alpha to gamma crystallin in US and UI exists in 6 week old rat lens cortex and nucleus as well. Separations of US and UI on Sephadex G 100 chromatography have indicated similar relationships between gamma and alpha crystallins in the insoluble subractions. The presence

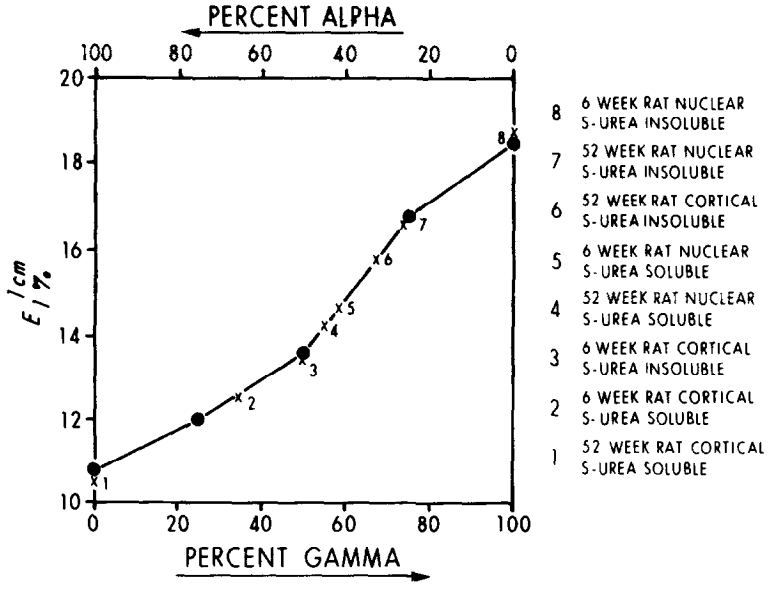

Figure 5. Ultraviolet analysis of alpha and gamma crystallin levels in rat lens nucleus and cortex. Points (closed circles) were obtained by calculating the $E^{1cm}_{1\%}$ values at 280 mμ of mixtures of purified S-alpha and S-gamma crystallins. The US and UI extinction values are marked along the curve with x's.

of both alpha and gamma crystallin in the US and UI fractions was also shown by immunoelectrophoresis (Figure 6).

Sclerosis of the nucleus of the lens appears to be a two step process whereby the lens soluble proteins are first converted into a weakly associated loose gel in the cortical region of the lens. With aging, the cortex is compressed into the nucleus where the loosely associated gel is converted into a firm tight gel. The loose gel of the cortex is composed mainly of the urea-soluble fraction, and seems to be made up of approximately equal levels of alpha and gamma crystallins held together by hydrogen and hydrophobic attractions. The tight gel of the nucleus, mainly urea-insoluble protein, consists mainly of gamma crystallin and is held together partly by SS bridges. Formation of the firmly-bound gel structure

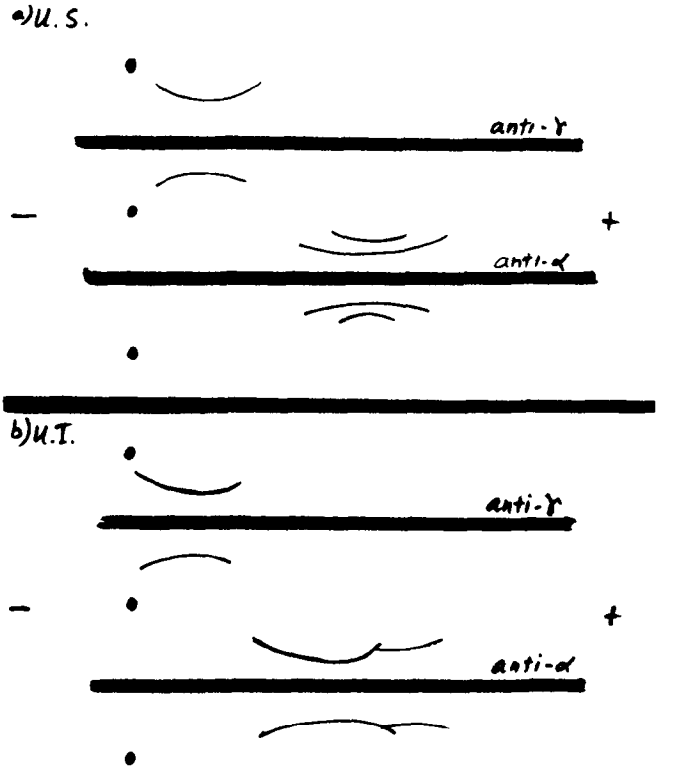

Figure 6. Immunoelectrophoretic analysis on 1% agarose of a) US and b) UI fractions of rat lens proteins. Antisera to alpha and gamma crystallins were prepared by subcutaneous injections of DEAE-cellulose-purified antigens into rabbits in Freund's adjuvant.

seems to depend upon the establishment of the weakly bound gel first, so that groups which must combine to form the firm gel can interact with each other as time passes, or as other forces bring them into proximity. Factors which may be involved in the conversion of the US to UI could include: the exclusion of water surrounding reactive groups; the exclusion of SH masking or exchanging molecules, such as glutathione which is known to be present at higher levels in the lens nucleus (9); a change in folding of protein molecules which exposes more reactive groups due to pressure on the protein gel in the nucleus resulting from decreased lens capsule elasticity and increasing lens volume.

The discussion above is consistent with the idea that gamma crystallin is the soluble protein whose properties predicate urea-insoluble protein formation. It has the greatest SH content of all the soluble lens proteins; it seems to possess a high S$^{\cdot}$ free radical content even without previous irradiation (10), it is present in the nucleus at much higher levels than in the cortex; its structure (11), (i.e; low helical content) and high apolar amino acid content would enable easy association with alpha crystallin and with itself to form aggregates. Studies have been reported in which gamma crystallin aggregates to insoluble products as a result of the oxidation of SH to SS (12,5). It is also shown in this report that with aging the level of gamma crystallin in the soluble phase of the lens is markedly depressed (10). All of these facts emphasize the importance of gamma crystallin in the process of nuclear sclerosis. Further importance of gamma crystallin in nuclear sclerosis is illustrated in the chicken lens, which has no gamma crystallin, low SH content, and no hard central nucleus, even in old age. Recent studies indicate that only trace amounts of UI are present in even the old chicken lens.

This scheme of nuclear sclerosis applies perfectly well to the human lens. The work of Pirie (13) and of our group (14) indicates that the urea insoluble portion of the water insoluble protein of human lenses also contains the gamma crystallin which appears to be lost from the soluble phase of the lens with aging. In the human lens, however, a much higher alpha crystallin content has been found in both

the urea soluble and urea insoluble portions of the water insoluble protein than in the rat. This further emphasizes the quantitative rather than qualitative differences between the composition of the insoluble protein of the lenses of different species, and the relationship between the insoluble fraction and soluble protein distribution. In the human lens other types of bonding between protein molecules which are not yet fully characterized appear to be as important as the formation of SS bridges. These other types of bonding are probably between oxidized phenolic groups of the tyrosine residues which are found in abundance in gamma crystallin. Such oxidative products seem to cause the yellow to brown coloration of human lenses.

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