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Protein-Protein Interactions and Lens Transparency

Larry Takemoto[a,*] and **Christopher M. Sorensen**[b]

a*Division of Biology, Kansas State University, Manhattan, KS 66506, USA.*

b*Department of Physics, Kansas State University, Manhattan, KS 66506, USA.*

Abstract

Past studies have identified posttranslational modifications of human lens proteins occurring during cataract formation, and have also demonstrated that protein-protein interactions exist between different lens crystallins. Based upon current theories of lens transparency, these posttranslational modifications and their possible effects upon crystallin interactions may be the key to understanding why the lens is able to transmit light, and why transmission is decreased during cataractogenesis. This review will summarize current knowledge of posttranslational modifications during human cataractogenesis, and will propose their possible role in protein-protein interactions that are thought to be necessary for lens transparency. Based upon this premise, model systems will be described that will test the validity of the theory.

1. Introduction

The lens is comprised of a very high concentration of protein (approximately 300 mg/ml), that is necessary for the refractile properties of the tissue. In spite of this, and because of this very high protein concentration, the newborn human lens is completely transparent. During aging, the lens slowly loses some of its transparency, then loses more transparency during development of the senile cataract. In a small percentage of patients with congenital cataract, opacification occurs at a much younger age, due to genetic mutation.

In order to fully understand the molecular mechanisms involved in lens opacification, it is first necessary to understand why a tissue of such high protein concentration is transparent. The protein composition of mature lens fiber cells is comprised of three general classes of proteins; alpha crystallins, beta crystallins, and gamma crystallins (Bloemendal, 1977). The alpha crystallins are comprised of two highly related sequences, the beta crystallins are mainly comprised of six highly related sequences, and the gamma crystallins are mainly comprised of three highly related sequences (Lampi et al., 1997). In the human lens, the alpha crystallins form oligomers of large size, the beta crystallins form oligomers of smaller size, and the gamma crystallins exist as monomers (Bloemendal, 1977).

Why is the lens with such a high concentration of protein transparent? Trokel (1962) suggested that a “paracrystalline state” of these proteins minimized light scattering, and resulted in the lens transparency. However, Benedek (1971) predicted that a paracrystalline array was not necessary, and that a limited degree of short-range order due to repulsive, nearest-neighbor

*Corresponding author. Division of Biology, Kansas State University, Manhattan, KS, 66506, USA. Tel: +1 785 532 6811; fax: +1 785 532 6799. *E-mail address:* takemlj@ksu.edu.

interactions at high protein density was sufficient for transparency. In a classic study, Delaye and Tardieu (1983) used small angle X-ray scattering to demonstrate the absence of protein spatial order in concentrated solutions of lens proteins, to show that liquid-like, short-range order of proteins and the consequent lack of large scale fluctuations were sufficient to explain the transparency of the lens.

2. Short-range order involves interactions of crystallins

Tardieu et al. (1992) used X-ray scattering and osmotic pressure studies to demonstrate interactions amongst gamma crystallins, a finding that was confirmed by Stevens et al. (1995) using NMR. Based upon light scattering studies, Bettelheim and Chen (1998) suggested that there also existed interactions between different classes of crystallins, with alpha-gamma interactions being the strongest. Additional studies using NMR (Carver et al. 1994), light-scattering (Thurston et al. 1999), two-hybrid analysis (Fu and Liang, 2003), filtration (Biswas and Das, 2004), and microequilibrium dialysis (Ponce and Takemoto, 2005) all confirmed the existence of crystallin interactions.

In a recent study (Stradner et al., 2007), neutron scattering and molecular dynamics simulations were used to study the possible role of protein interactions in the transparent properties of the lens. The results strongly suggested that weak, but neither zero nor strong interactions between alpha and gamma crystallins play an important role in lens transparency. Their explanation used a gamma modulated depletion interaction between the alpha crystallins when the attractive interactions were zero or very weak. A depletion interaction occurs in a mixture of hard spheres of different sizes (Mendez-Alcaraz and Klein, 2000). Consider the fact that a small sphere cannot get closer than its radius to a larger sphere. Thus associated with each larger sphere there is a depletion layer around its surface with thickness equal to the small sphere radius. The larger spheres can increase the free volume available to the more numerous smaller spheres by being close enough to mutually exclude the smaller spheres in the region between the larger spheres. This increase in free volume causes an increase in entropy of the small sphere system which is thermodynamically favorable. Thus the larger spheres are attracted to each other. This depletion interaction will be destroyed if the attractive interaction between the different sized spheres becomes more than very weak. Hence, Stradner et al., 2007 proposed weak interaction between gamma and alpha crystallins is necessary to deaggregate the alpha crystallins from each other. However, too strong of a gamma crystallin-alpha crystallin attraction will cause gamma crystallin-alpha crystallin aggregates which will scatter light and decrease transparency.

3. Weak interactions between lens crystallins change during aging/ cataractogenesis

If these interactions are important in lens transparency, is it possible they change during lens aging and/or cataractogenesis? Surface plasmon resonance demonstrated an increase interaction of alpha crystallins with a covalently modified form of gammaB crystallin found in aged bovine lens (Peterson et al., 2005), while microequilibrium dialysis showed that some interactions of alpha and gamma crystallin actually decreased during aging (Takemoto and Ponce, 2006; Takemoto et al., 2008). Using a two-hybrid system, it was possible to study the possible interactions of mutant forms of alpha and gamma crystallins that are thought to cause human congenital cataracts. The results demonstrated that in most cases, mutation of either alpha or gamma crystallin resulted in a decrease in their interaction (Fu and Liang, 2003).

A change in crystallin interactions, whether between the same crystallins or between different crystallins, could result spatially in non-uniform changes in protein density, resulting in non-uniform refractive index and subsequent increase in light scattering. As a test of this hypothesis,

Fourier analysis of stained lens sections has been used as an indicator of protein density changes in the normal and cataractous lens. Vaezy et al., 1995 showed that relative to normal lens, there were changes in spatial fluctuations in stained sections from cataracts of selenite-treated rats. Changes were also seen in advanced nuclear cataracts from humans, when compared with age-matched clear lenses (Metlapally et al., 2008).

Figure 1 summarizes the possible causes and involvement of protein-protein interactions in loss of lens transparency. During normal aging, posttranslational modifications of lens proteins result in altered protein-protein interactions, resulting in fluctuations in protein density. Previous studies have indeed shown an age-related decrease in the interactions of alpha crystallins and some of the gamma crystallins (Takemoto et al., 2008). These, and possibly other changes, accumulated during aging result in a gradual decrease in the observed transparent properties of the lens (Bron et al., 2000). During senile cataractogenesis, increased posttranslational modifications of the same type and/or introduction of new chemical modifications cause increased changes in protein density fluctuations, resulting in significant loss of transparency and cataract. In human congenital cataracts, expression of specific genetic mutations of crystallin genes cause changes in protein-protein interaction and subsequent protein density fluctuations, but at a younger age.

4. Covalent changes during human cataractogenesis

The noncovalent, weak interactions of lens proteins discussed in the previous section could be perturbed by covalent changes of the lens proteins themselves. Such covalent changes may play an initiating role in the development of opacification. A summary of known covalent changes and their possible physiochemical consequences would help clarify the exact biochemical mechanism(s) involved in cataractogenesis. In human congenital cataracts occurring at an early age, the chemical change resulting in loss of transparency is the mutation in the wild type sequence. Table 1 lists the known mutations found in human genetic cataracts, together with the reported change in protein when expressed in a recombinant system. In almost all cases, the expressed mutant protein is involved in aggregation, which may manifest itself as a change in water solubility. If they occur *in vivo*, such aggregates will result in changes in protein density. In some cases, formation of aggregates of sufficient size (i.e $>50 \times 10^6$ daltons) can directly scatter light (Benedek, 1971). However, as long as the aggregates do not change protein density and do not directly scatter light, they will not result in loss of lens transparency.

Since most cataracts occur in older patients, and since there is no strong evidence linking mutation of specific genes as causative events in this process, posttranslational modifications probably play the most important role in cataractogenesis. Table 2 lists the specific covalent changes found in multiple human senile cataracts, that are not found at all, or found in smaller amounts in lenses from normal age-matched patients. Many other posttranslational modifications have been reported in human cataracts, but additional studies need to done to verify that these changes, either qualitatively or quantitatively, are unique to human cataracts. All modifications except one involve oxidation of either methionine, cysteine, or tryptophan residues. In relation to this review, oxidation of cysteine is particularly noteworthy, since oxidation of this residue to half-cystine can result not only in intramolecular crosslinking, but also in intermolecular crosslinking that would result in a change in protein-protein interactions. This is especially relevant to some of the gamma and beta crystallins, which contain a relatively high concentration of cysteine residues in their sequence, that can potentially form intermolecular disulfide bonds in the presence of oxidizing agents.

Also noteworthy is the deamidation of asn-143 of gammaS crystallin. Deamidation of asn or gln residues to asp or glu is the most prolific posttranslational modification occurring in crystallins of the aging normal human lens (Wilmarth et al., 2006; Hains and Truscott, 2007).

It results in the introduction of a negative charge that may have profound effects upon protein-protein interaction. In other tissues of the body, deamidation has been implicated in aggregation of the prion protein in Creutfeldt-Jakob disease (Qin et al., 2000), tau protein in Alzheimer's disease (Watanabe et al., 2004), and the hormone amylin in diabetes (Nilsson et al., 2002). In the lens, deamidation has been shown to cause structural changes in alphaB crystallin (Gupta and Srivastava, 2004) and betaB1 crystallin (Lampi et al., 2001), that may lead to eventual aggregation.

5. Involvement of protein-protein changes in lens opacification

Based upon studies described in this review, Figure 2 shows the possible involvement of the magnitude of protein-protein interactions upon lens opacification. In the normal transparent lens [B], weak interactions between heterologous crystallins such as alpha and gamma crystallins ensure a uniform protein density across the lens, resulting in lens transparency. During aging of the normal lens [A], a decrease in these interactions, possibly to zero (Takemoto and Ponce, 2006;Takemoto et al., 2008), results in an increase in homo-oligomeric interactions such gamma-gamma as well as the gamma driven depletion interaction of the alpha crystallins. Formation of homo-oligomeric complexes with relatively high protein density, also results in regions of relatively low protein density, with subsequent protein density fluctuations. During cataractogenesis [C], an increase in strong protein-protein interactions causes formation of aggregates, which result in regions of higher protein density fluctuation and/or aggregates of sufficient size to directly scatter light.

6. Other causes of lens opacification

Although this review hypothesizes that alterations in crystallin interactions play a major role in opacification, it is possible that other biochemical/biophysical mechanisms are also important in loss of lens transparency. Alterations in protein-protein interaction may not only involve crystallins, but may also involve non-crystallin components. The uniformity in lens protein density could be stabilized by the presence of cytoskeletal proteins, which have been shown to bind to crystallins, and which have been shown to decrease in selenite cataracts in rats (Clark et al., 1999). Recent studies have shown that the R116C mutation of alphaA crystallin causes congenital cataract in humans (Cobb and Petrash, 2000), and exhibits decrease binding to the cytoskeletal component actin *in vitro* (Brown et al., 2007). In addition, the R120G mutation in alphaB crystallin responsible for human cataract shows altered interaction *in vitro* with an intermediate filament protein of the lens (Perng et al., 2004; Song et al., 2008).

Movement of water from a protein-bound to a protein-free state (i.e. syneresis), and a temperature dependent redistribution of water and protein (i.e. phase separation) could also result in density fluctuations. Bettelheim and co-workers measured the non-freezable (protein bound) and freezable (unbound) water content of normal and cataractous human lenses, and found that in the cortex and nucleus of cataractous lenses there was a greater freezable water content than control lenses (Bettelheim et al., 1986a). Increased syneresis was also found in opaque versus transparent regions of the same lens (Bettelheim, et al., 1986b). Since separation of phases is dependent upon temperature, any protein mutation resulting in phase separation at physiological temperature will result in cataract such as found for the R14C mutation of gammaD crystallin, which causes a hereditary cataract in humans (Pande et al., 2000). Compounds that inhibit the separation of phases *in vitro*, have been shown to be effective in inhibition of cataract *in vivo* (Clark and Steele, 1992).

7. Test of hypothesis: Future Studies

Based upon the figures and tables of this review, we hypothesize that posttranslational modifications or genetic mutation result in change in protein-protein interactions between lens

crystallins, which result in a decrease in lens transparency. With available methodology, it should be possible to rigorously test this hypothesis.

First, a more systemic characterization should be done of posttranslational modifications specific to the human cataractous lens. The continuing development of more sensitive and accurate mass spectrometers should greatly assist in this study. Posttranslational modifications that are either unique, or found in greater amount, should be identified in cataractous lenses as compared with age-matched normal lenses. With the characterization of multiple cataractous lenses, it should be possible to determine if there are unique classes of posttranslational modifications (and residues) that are preferentially found in the cataract lenses, or whether cataractogenesis is simply an increased degree of posttranslational modification that already manifests itself in the aging normal lens.

Next, many of the crystallins with these changes, together with an increasing number of genetic mutants that cause congenital cataracts, can be expressed in a recombinant system. The purified proteins can then be used to determine possible changes of weak protein interactions *in vitro*, using any of the methodologies previously described in Section 3 of this review.

At the same time, the effect of these changes can be assessed *in vivo*, using both knock-out and knock-in strains of animals. Knock-out of both the alpha crystallin genes has already shown to result in formation of cataracts (Boyle et al., 2003), and knock-in of the alphaB crystallin R120G mutation found in human congenital cataracts resulted in lens opacity (Andley et al., 2008). Electron microscopy of lens sections (Vaezy et al., 1995; Metlapally et al., 2008) and scattering studies of lenses *in vivo* (Bursell et al., 1989; Simpanya et al., 2005) could verify whether knock-out of alpha crystallin genes result in fluctuations in protein density. After knocking-out certain crystallin genes, the genes could be replaced in a knock-in animal with the mutated or posttranslationally modified crystallin, found in human cataracts. Together with the *in vitro* studies, formation of lens opacity, as well as changes in protein density and/or aggregate formation *in vivo* would strongly support the hypothesis that covalent changes in lens crystallins cause alterations in protein-protein interactions that eventually result in cataractogensis.

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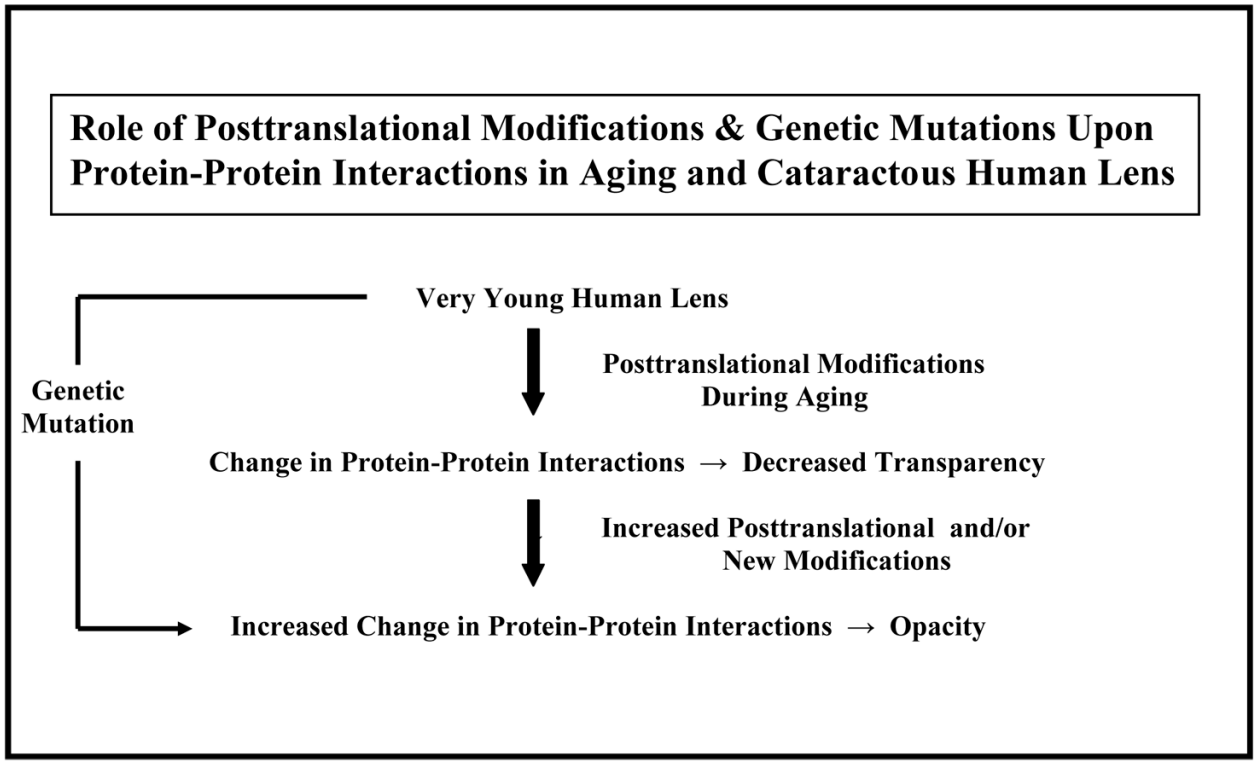

Figure 1.
Role of posttranslational modification and genetic mutation upon protein-protein interactions in the aging and cataractous human lens. A very young transparent lens undergoes posttranslational modifications during aging (thick arrow), resulting in change in protein-protein interactions and subsequent decrease in transparency. During cataractogenesis, increased posttranslational modifications cause increased protein-protein interactions (thick arrow), eventually resulting in increased loss of transparency. In congenital cataract (thin arrow), mutation of crystallins causes increases in protein-protein interaction over a much shorter time period.

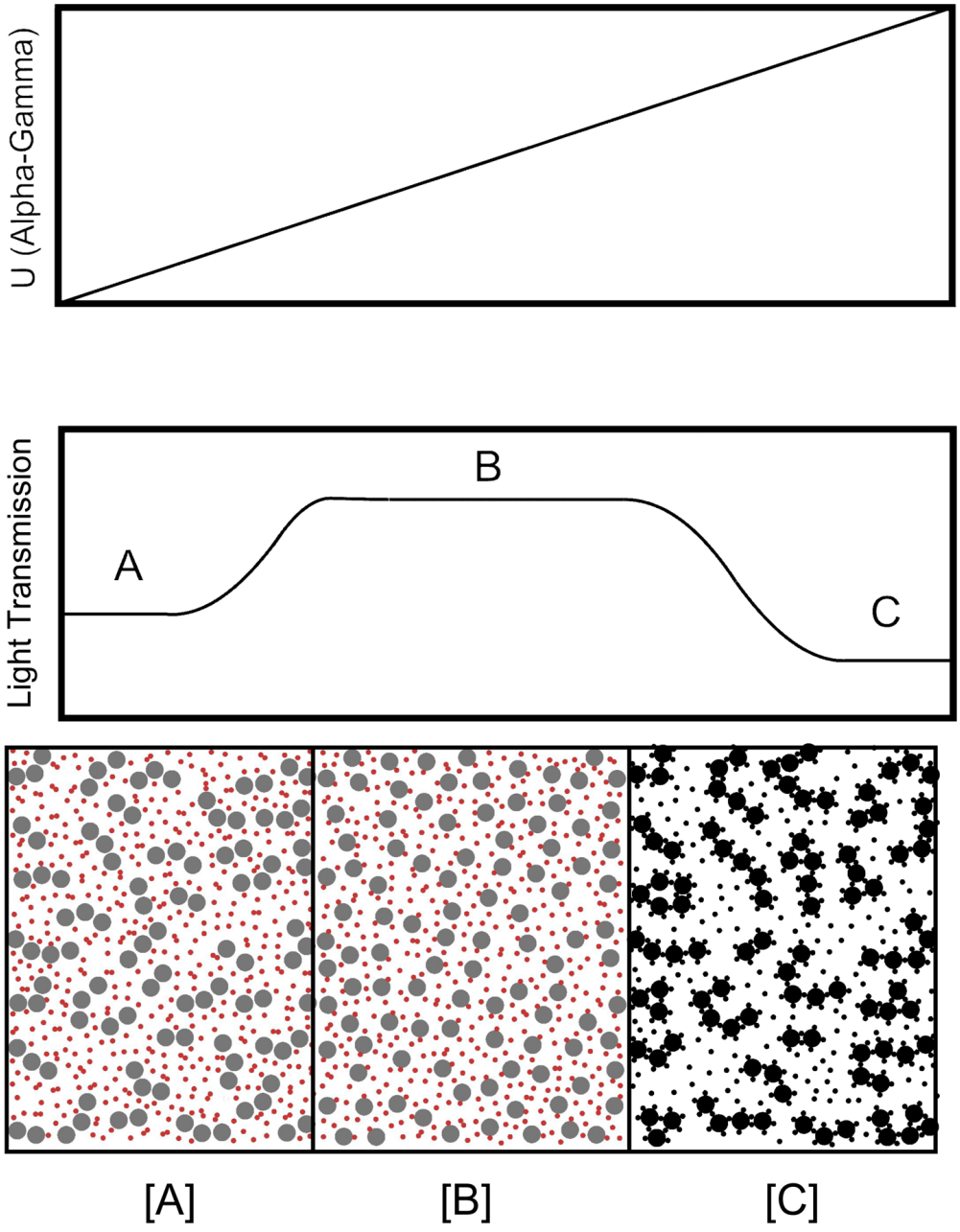

Figure 2.

Role of attractive alpha-gamma interactions on light transmission in the lens. Upper panel plots the interaction potential, U, increasing to the right from zero. Middle panel sketches the qualitative nature of the light transmission as the interaction potential changes from A, modest light transmission when the interaction is small to B, good light transmission for an intermediate amount of interaction to C, poor light transmission when the interaction is large. The lower panel sketches the molecular arrangement for A, B, and C. Here the large spheres represent alpha crystallins, the smaller spheres the gamma crystallins. Subpanel [A] shows a modest depletion interaction (Stradner et al., 2007) (note there are no gammas between the closely neighboring alphas) hence some modest alpha aggregation. These aggregates cause some

scattering so light transmission is modest but not optimal. In Subpanel [B] the alpha-gamma interaction is small but large enough to destroy the gamma modulated depletion interaction between the alphas. Thus there is no aggregation, hence little scattering which in turn implies high light transmission. In Subpanel [C] the alpha-gamma interaction has grown so large that it not only has destroyed the depletion interaction but also caused strong linkages between alphas and gammas and consequent strong aggregation. These aggregates scatter increased amounts of light, so light transmission is low

Table 1

Mutations Causing Human Cataracts

Known mutations in human crystallin genes thought to cause congenital cataracts. The second column summarizes characterization of the expressed mutant protein that could possibly play a role in cataractogenesis. ND, not determined

Mutation	Protein Change	Reference
alpha (R21L)	ND	Graw et al., 2006
alphaA (R49C)	ND	MacKay et al., 2003
alphaA (G98R)	Aggregation	Murugesan et al., 2007
alphaA (R116C)	Increased membrane binding, decreased actin binding, change in heterologous crystallin binding	Cobb and Petrash, 2000 Fu and Liang, 2003 Brown et al., 2007
alphaB (P20S)	ND	Liu et al., 2006a
alphaB (R120G)	Aggregation	Treweek et al., 2005
alphaB (D140N)	Aggregation	Liu et al., 2006b
alphaB (450delA)	ND	Berry et al., 2001
betaA1/A3 (G91del)	Aggregation	Reddy et al., 2004
betaB1 (G220X)	Water insolubility	Mackay et al., 2002
betaB1 (C-terminal elongation	ND	Willoughby et al., 2005
betaB2 (Q155X)	ND	Li et al., 2008a
gammaC (R168W)	AggregationTalla et al., 2006	
gammaD (R14C)	Aggregation via disulfide bonding	Pande et al., 2000
gammaD (P23T)	Change in solubility	McManus et al., 2007
gammaD (G61C)	ND	Li et al., 2008b
gammaD (R58H), gammaD (R36S)	Decreased solubility	Pande et al., 2001
gammaD (E107A)	ND	Messina-Baas et al., 2006
gammaD (W156X)	ND	Santhiya et al., 2002
gammas (G18V)	ND	Sun et al., 2005

Table 2

Posttranslational Modifications Associated With Human Senile Cataracts

Posttranslational modifications associated with human senile cataracts. The modifications are either not found in age-matched normal lenses, or are found in lower amount in age-matched normal lenses.

Modification	Reference
Oxidation of various methionine residues (residue number unknown)	Garner and Spector, 1980
Oxidation of cys-131 and cys-142 of alpha crystallin	Takemoto, 1996
Oxidation of cys-37 and cys-66 of betaB2 crystallin	Takemoto, 1997a
Oxidation of cys-170 and cys-185 of betaA3/A1 crystallin	Takemoto, 1997b
Deamidation of asn-143 of gammaS crystallin	Takemoto and Boyle, 2000
Oxidation of tryptophan of various crystallins	Hains and Truscott, 2007