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Carnitine protects the molecular chaperone activity of lens α-crystallin and decreases the posttranslational protein modifications induced by oxidative stress

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ABSTRACT

α-Crystallin prevents heat-induced lens protein aggregation by acting as a molecular chaperone. We investigated whether oxidative stress in an in vitro model of cataract increases the posttranslational modifications to lens proteins induced by transglutaminase (TGase). We also investigated whether carnitine safeguards the chaperone activity of α-crystallin and inhibits the TGase-mediated aggregation of lens proteins and lens opacification. One lens of each pair of rat lenses was cultured without H_2O_2 (controls), and the controlateral lens was exposed to 500 μM H_2O_2, an as oxidizing agent, in the presence and absence of 300 μM L-carnitine. Lenses treated with H_2O_2 alone became opaque, whereas the L-carnitine-treated lenses remained clear. Incubation with 500 μM H_2O_2 significantly increased the level of TGase-mediated cross-links of lens protein and decreased the ability of α-crystallin to prevent heat-induced aggregation of β_L-crystallin. L-carnitine prevented both these negative effects. After H_2O_2, glutathione and free carnitine levels dropped dramatically. L-carnitine did not prevent the loss of glutathione, but it did prevent carnitine from falling below control values. In conclusion, carnitine safeguards the chaperone activity of α-crystallin that prevents protein aggregation in the lens, and it decreases the protein posttranslational modifications induced by oxidative stress. This process is not mediated by glutathione.

Key words: α-crystallin • carnitine • cataract • lens proteins • oxidative stress

The ocular lens is a transparent organ constituted by a highly concentrated and highly ordered matrix of structural proteins, called “crystallins,” which are probably the longest lived proteins of the body (1–4). Posttranslational modifications of lens crystallin, consequent to aging or diseases such as diabetes, may result in conformational changes and aggregation of these proteins and lead to lens opacification and cataract formation (5). Although

the mechanisms of cataractogenesis are not well understood, oxidation of lens proteins is associated with cataract in mammals (6).

The lens undergoes major oxidative stress because it is constantly exposed to light and oxidants (7–12). In fact, the stressed lens is subject to selective oxidation of specific amino acids that results in charge alterations, protein degradation, cross-linking, insolubilization, and increased nontryptophan fluorescence (13–17). The lens has evolved antioxidant systems and repair mechanisms to counteract the effect of oxidants. The first line of defense against oxidation stress is constituted by radical scavenging antioxidants that reduce the oxidative insult. For example, glutathione (GSH) and taurine, which are both highly represented in lens tissue, exert protective effects in an in vitro model of diabetic cataract (18, 19). Furthermore, α-crystallin, which constitutes up to 50% of the total protein mass of the mammalian lens, acts as a molecular chaperone that prevents heat-induced aggregation of numerous proteins and is required for the renaturation of chemically denaturated proteins (20–22). A key element of α-crystallin function is its ability to prevent aberrant protein associations by binding to transiently exposed hydrophobic protein surfaces (23). Because α-crystallin prevents both ultraviolet-radiation- and free-radical-induced aggregation of proteins in vitro (24–29), it may also protect lens proteins from photooxidative changes in vivo.

The discovery of transglutaminase (TGase) activity in the lens of mammals and of significant amounts of γ-glutamyl-ε-lysine isopeptide bonds in cataractous lenses led to the suggestion that TGase-mediated changes can be important in the aging of the lens and in cataractogenesis (30, 31). TGases are a group of Ca^{2+}-dependent enzymes involved in the formation of ε-(γ-glutamyl) lysine isopeptide cross-links in polypeptides and in the conjugation of amines to certain glutamine residues in proteins. Exposure of crystallins to oxidizing free radicals also enhances their susceptibility to TGase (32).

We previously showed that in experimental animal diabetes the decrease in lens carnitine, a ubiquitous molecule involved in many biological pathways, is an early important and selective event possibly related to cataract formation (33). In addition to its primary function as a carrier of long-chain fatty acids from the cytoplasm to the sites of β-oxidation, carnitine could also serve to maintain cell homeostasis (34). For example, protein sites that are most readily susceptible to posttranslational modifications could be primary targets of the direct or indirect acetylation process induced by L-carnitine (35). These considerations explain why carnitine levels in various animal tissues do not invariably correlate with tissue energy requirements or with lipid metabolism. For example, the eye lens, a nonvascularized tissue whose main source of energy is glucose absorbed from ocular fluids, has higher carnitine concentrations than other eye compartments (36).

The aim of this study was to evaluate whether the addition of L-carnitine to an in vitro model of cataract protects the chaperone activity of α-crystallin and inhibits both TGase-mediated aggregation of lens proteins and lens opacification.

MATERIALS AND METHODS

Lens organ culture

Four-month-old Sprague-Dawley rats were anesthesized with 5 mg/kg xylazine and 65 mg/kg ketamine and decapitated. Immediately after, eyes were enucleated, extracted, and placed in 2 ml of modified TC-199 medium. Lens integrity was assessed by measuring protein leached into the medium after 30–60 min of culture; damaged lenses were discarded. One lens of each pair was placed in culture medium with no H_2O_2 and used as a control. After 24 h of culture, the control lenses did not differ from freshly enucleated lenses in any of the parameters evaluated in this study.

The controlateral lens of each pair was placed in medium and, after equilibration under 5% CO_2 at 37°C, was exposed to 500 μM H_2O_2 in the absence or presence of 300 μM L-carnitine. After 24 h of incubation, morphological characteristics and changes were recorded and the lenses were photographed. The incubated lenses were rinsed with saline solution, blotted on filter paper, weighed, and immediately processed for biochemical analysis. To determine lactate dehydrogenase (LDH) leakage, lenses were incubated individually in each medium tested, and the medium was harvested daily and saved for LDH analysis.

Extraction of lens proteins

Decapsulated lenses were homogenized with disposable pestles and then sonicated in extract buffer (20 mM HEPES, 0.2 mM EDTA, 0.5 mM dithiothreitol, 450 mM NaCl, 25% glycerol, 0.5 μg/ml leupeptin, 0.5 μg/ml protinin, 0.5 mM phenylmethaesulfonyl fluoride) on ice. Aliquots of the homogenate from each of the incubated lenses were removed for GSH and L-carnitine analysis. The remainder was centrifuged for 25 min at 20,000 x g to separate the supernatant from the pellet. The pellet was washed with 1.0 ml buffer and dried under nitrogen. This fraction was designated the “water-insoluble” fraction. The supernatant fraction was dialyzed twice against 3 ml of 0.025 mol/l phosphate buffer, pH 7.4, for 48 h and lyophilized. This fraction was designated the “water-soluble” fraction. The water-soluble and water–insoluble fractions were delipidated with 3.0 ml chloroform:methanol (2:1) for 16 h under shaking followed by centrifugation at 2000 g for 5 min. After the organic solvent was discarded, the residue was treated with 2.0 ml of diethyl ether, left to stand for 5 min, and then centrifuged at 2000 g for 5 min. The pellet was dried under air and stored at 4°C in a desiccator.

Preparation of crystallins

The water-soluble crystallin fractions were isolated by preparative Sephacryl S-300-HR gel permeation chromatography as previously described (29, 37). In brief, soluble protein was applied to a 100- × 1.5-cm column and developed isocratically with phosphate buffer. The total fractions from control lenses and lenses treated with H_2O_2 ± L-carnitine were concentrated by ultrafiltration in a Diaflo apparatus, and their purity was checked by SDS-PAGE, done according to Laemmli by using a Bio-Rad Mini-Protean II System. Protein concentrations were measured with a Bradford protein assay kit (Bio-Rad, Hercules, CA).

Western blotting

Total lens homogenate was applied on 4–20% gradient sodium dodecyl sulfate (SDS) gels by using Tricine buffers and was then transferred to polyvinylidene difluoride membranes. Western blotting was performed as described elsewhere (38). The concentration of antibodies was 5 μg/ml for primary antibody (anti-γ-glutamyl-ε-lysine isopeptide) and 0.1 μg/ml for secondary antibody. The blot was then developed by enhanced chemiluminescence (Pierce, Milan, Italy). Subsequently, the very high molecular weight bands were cut out, eluted into SDS buffer containing Tricine, freed of SDS by ion pair extraction (39), and subjected to amino acid analysis.

Measurement of isopeptide cross-links in water-insoluble proteins

The water-insoluble proteins were suspended in 0.2 M *N*-ethylmorpholine acetate (pH 8.1). An aliquot (10%) was used to measure the amount of total protein. Samples were digested by the sequential addition of proteolytic enzymes (collagenase, pronase, aminopeptidase and carboxypeptidase A, carboxypeptidase B, and carboxypeptidase y) directly to the reaction mixture at 37°C in the presence of 0.02% sodium azide. After enzymatic digestion, the free N^{ε}-(γ-glutamyl)lysine isopeptide cross-link was resolved by HPLC and quantified by amino acid analysis (40). In a related set of experiments, the isopeptide content of lenses was determined without prior extraction.

Tryptophan fluorescence

The loss of protein tryptophan fluorescence, an indicator of tryptophan oxidation, seems to be a marker of crystallin integrity. We therefore measured tryptophan fluorescence in lens crystallin (Perkin-Elmer 650-40 spectrophotometer) according to a previously described method (41). The excitation wavelength was set to 295 nm, and the fluorescence emission was detected at 330 nm.

Evaluation of the molecular chaperone activity of α-crystallins from control and in vitro treated lenses

The following experiments were performed essentially as described elsewhere (42). The chaperone-like activity of α-crystallin from control lenses and lenses treated with $H_2O_2 \pm$ L-carnitine was determined by heat denaturation studies. The extent to which the unmodified or modified α-crystallin preparation protected β_L-crystallin (used as the target protein) from heat-induced denaturation and aggregation was assessed as follows: 100 μg or 200 μg of α-crystallin were added to 200 μg of β_L-crystallin in a 1.5-ml cuvette and made up to a final volume of 1 ml with 50 mM phosphate buffer, pH 7.0. The cuvette was placed in a temperature-regulated cell holder attached to a ultraviolet spectrophotometer. Light scattering due to protein denaturation and aggregation was monitored at 360 nm absorbance for 3000 s at 55°C or for 1800 s at 58°C.

Intermediate filament assembly and viscosity assays involving α-crystallins

The sedimentation assay devised by Nicholl and Quinlan (43) was used to assess α-crystallin-induced inhibition of intermediate filament assembly. Purified porcine glial fibrillary acidic protein (GFAP) was used for these studies; it was purified from porcine spinal cord by axonal flotation as described previously (43, 44). The gel formation assay was based on a method used

to monitor actin binding protein activity by falling ball viscosimetry (45). α-Crystallins were mixed with GFAP in 8 M urea; 20 mM Tris-HCl, pH 8.0; 5 mM EDTA; and 25 mM 2-mercaptoethanol and were then stepwise dialyzed in 10 mM Tris-HCl, pH 8.0, and 25 mM 2-mercaptoethanol. Assembly of the GFAP intermediate filaments, in the presence or absence of α-crystallin, was induced by the addition of a 20-fold concentrated buffer to give a final concentration of 100 mM imidazole-HCl, pH 6.8, 0.5 mM DTT. A 100-μl aliquot of sample was loaded into a glass tube and used for the viscosity assay. The tube was then immersed in a 37°C water bath for 1 h before the gel formation assay. A ball was then placed in the tube, and the ability of the solution to support the ball was monitored.

Lens microscopic examination

After a 24-h incubation with or without H_2O_2 in the presence or absence of L-carnitine, lenses were submitted to standard procedures for histologic analysis. For optical microscopy, lenses were removed from culture medium, immersed in fixative (neutral buffered formalin), dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. Five-micrometer sections were prepared and stained with hematoxylin and eosin. For scanning electron microscopy the lenses were fixed by immersion for at least 24 h at room temperature in a solution of 2.5% glutaraldehyde and 6% sucrose, buffered to pH 7.2 with 50 mM sodium cacodylate. Samples were dehydrated through a graded series of ethanol, critical point-dried using CO_2, mounted on aluminium stubs, sputter-coated with gold, and examined with a Leica Stereoscan 440 microscope at a 3–7 kV acceleration voltage. For transmission electron microscopy, the lenses were fixed as described previously for the scanning electron microscopy procedure, postfixed in OsO_4, buffered with 150 mM sodium-potassium phosphate (pH 7.4), embedded, sectioned, and stained for electron microscopy. They were examined using a JEOL 100B electron microscope.

Statistical analyses

Results are reported as the mean ± SD, unless otherwise specified, of at least three separate experiments. Statistical significance of differences was examined using an independent *t* test or one-way ANOVA as required. Post hoc multiple comparisons were performed using Student-Newman-Keuls tests. Intergroup comparisons were made with by a Mann-Whitney U test.

RESULTS

Changes in lens morphology

After 24 h of incubation, control lenses remained clear, whereas lenses exposed to 500 μM H_2O_2 were swollen and the entire outer cortical region was uniformly cloudy. After 24 h of incubation, H_2O_2-treated lenses were significantly heavier than control lenses (Table 1). There were no differences in weight between control lenses and lenses treated with both L-carnitine and H_2O_2. The lenses treated with H_2O_2 alone became opaque, whereas lenses treated with L-carnitine and H_2O_2 remained clear. Optical and electron microscopy showed that cell shape was unaltered and that fiber cells were intact in control lenses and in lenses treated with L-carnitine and H_2O_2 (Fig.

1A, C, E). Ballooning, liquefaction, and various degrees of fiber swelling were observed in lenses exposed to H_2O_2 alone (Fig. 1B, D, F).

Free carnitine and GSH concentrations in control and H_2O_2-treated lenses

Free carnitine and GSH concentrations were unchanged in control lenses, whereas treatment with 500 μM H_2O_2 caused a precipitous drop in both GSH and free carnitine (Table 1). The addition of L-carnitine (300 μM) to the lens incubation medium before H_2O_2 treatment did not prevent the loss of GSH but maintained the free carnitine concentration almost at the level found in control lenses. To determine whether the decrease of free carnitine and GSH was related to lens damage, we measured leakage of LDH into the medium. As expected, after H_2O_2 treatment, the decrease in GSH and L-carnitine levels was accompanied by a significant increase of LDH in the supernatants, indicating that depletion of these factors was indeed associated with lens damage. In lenses treated with L-carnitine and H2O2, the concentration of GSH decreased to about the same level as in lenses exposed to H_2O_2 alone, but the concentration of LDH in the medium from the lens treated with L-carnitine and H_2O_2 was similar to that observed in control lenses. This indicates that L-carnitine can withstand this concentration of H_2O_2. It is of interest that the addition of L-carnitine to the lens incubation medium not only normalized the level of free carnitine in lens tissue treated with H_2O_2, but also produced a significant rise in the level of acetyl-L-carnitine.

Recovery of high molecular weight proteins in the water-insoluble lens fractions containing isopeptide cross-links

Water-insoluble proteins constituted only 5% of total proteins in control lenses, but increased to 41% of total proteins in H_2O_2-treated lenses (Table 1). The concentrations of water-insoluble proteins in lenses treated with L-carnitine and H_2O_2 approximated those observed in control lenses. To determine whether TGase activity contributed to the formation of water-insoluble protein deposits, we examined total lysates of lens specimens by using Western blotting and a highly specific anti-ε-(γ-glutamyl)lysine isopeptide antibody. Interestingly, very high molecular mass proteins were prominent in H_2O_2-treated lenses, but only minor immunoreactive products were seen in the control specimens and in lenses treated with both L-carnitine and H_2O_2 (Fig. 2).

Amino acid analysis revealed 1.3 cross-link residues/100 residues in the high molecular mass proteins eluted from the gel (Fig. 2, lane 4) and enzymatically digested. As controls for these experiments, we determined the numbers of cross-links in total lens extracts (water-soluble and water-insoluble proteins); these were 1 and 2 and 9–12 residues of cross-links/10,000 residues in control and H_2O_2-treated lenses, respectively. The latter value is consistent with the amount of insoluble proteins recovered from the H_2O_2-treated lenses, thus indicating that the higher levels of cross-links in these lenses originate from insoluble proteins. The values obtained with lenses treated with L-carnitine and H_2O_2 overlapped the control value. HPLC analysis of the proteolytic digest of water-insoluble lens proteins showed a relevant peak, corresponding to the isopeptide, only in lenses treated with H_2O_2 alone (Fig. 3).

Chaperone-like function of α-crystallin

The chaperone properties of the purified water-soluble α-crystallin were determined by β_L-crystallin (target protein) aggregation assay. Characteristically, β_L-crystallin aggregates at elevated temperatures. α-Crystallin, by exerting chaperone activity, either prevents or decreases the heat-induced aggregation of β_L-crystallin. Because the ratio of α to β determines the degree of protection against heat-induced aggregation, we used 100 μg or 200 μg of α-crystallin and 200 μg of β_L-crystallin. As expected, α-crystallin from control lenses exerted chaperone activity. H_2O_2 caused a significant decrease in the capacity of α-crystallin to prevent the heat-induced aggregation of β_L-crystallin, whereas L-carnitine prevented this negative effect. The chaperone-like activity of the water-soluble α-crystallin from control and treated lenses is illustrated in Figure 3.

Because intermediate filaments such as GFAP are a physiological target of α-crystallins, we tested α-crystallin chaperone activity by using falling ball viscosimetry in the gel forming assay (46). GFAP is an appropriate target because of the property of α-crystallin to disaggregate GFAP cytoplasmic inclusions. In the absence of α-crystallin, GFAP forms a protein gel that supports the ball used in the viscosity test. To determine whether H_2O_2 treatment affected the capacity of lens α-crystallin to disrupt the GFAP network, α-crystallin from control lenses or from lenses treated with H_2O_2 ± L-carnitine was added to the gel forming assay. α-Crystallin from control lenses completely inhibited the formation and maintenance of the GFAP gel in the viscosity assay, whereas α-crystallin from lenses treated with H_2O_2 alone did not affect gel formation (Table 2). In addition, α-crystallin from lenses treated with both L-carnitine and H_2O_2 blocked GFAP gel formation to the same extent as α-crystallin from control lenses.

Tryptophan fluorescence measurements

Tryptophan fluorescence was measured in α-crystallin fractions from control and treated lenses to identify conformational changes. In α-crystallin from H_2O_2-treated lenses, there was a 2.7-fold loss of tryptophan fluorescence; again, L-carnitine restored the control value (data not shown)

DISCUSSION

In this study, we have addressed whether L-carnitine protects the α-crystallin chaperone activity in lenses exposed to oxidative stress and reduces the posttranslational modifications of lens proteins. Exposure of lenses to H_2O_2 induced a significant reduction of L-carnitine content, decreased the solubility of lens proteins (the portion of water-insoluble proteins increased from 5% to 41%), and altered the chaperone activity of α-crystallin. Under the organ culture conditions used in this study 500 μM H_2O_2 was the threshold concentration at which morphological changes and protein modifications were detectable in the lens. GSH and free carnitine levels in the control lenses were similar to those measured in freshly enucleated lenses. This suggests that the organ culture conditions, at least those of the 24-h cultures, closely mimic the physiological environment of a healthy lens, although differences in the O_2 concentrations of organ culture and in vivo conditions cannot be ruled out.

Lenses exposed to L-carnitine and oxidative stress remained transparent. The protective effect of L-carnitine is not easily explained because L-carnitine per se is not known to exert antioxidant

activity, at least under the conditions used in this study. Neither did L-carnitine rescue GSH depletion, which means that the beneficial effect was not mediated by an increase of GSH through, for example, an anaplerotic effect on NADPH, a cofactor of the GSH reductase enzyme (47, 48). Rather, the fact that LDH leakage into the medium was not increased in lens treated with L-carnitine and exposed to oxidative stress indicates that the molecule can sustain lens integrity. This effect may be, at least in part, related to the capacity of carnitine and its acyl esters to repair the membrane phospholipids that are damaged by oxidative insult (49). Indeed, carnitine has been demonstrated to modulate the cellular acyl flux, which is critical for preservation of membrane structure and function (50).

We show that lens α-crystallin chaperone activity is diminished by in vitro oxidative stress and provide support for the proposal that lens proteins subjected to oxidative insult sustain a high degree of posttranslational modifications (32). L-carnitine not only reduced the increased posttranslational modifications of lens proteins but also afforded significant protection against the decreased chaperone activity of α-crystallin. α-Crystallin suppressed aggregation of denaturated proteins in studies in which mixtures of thermally stressed α-crystallins served as substrate (29). Oxidative stress disrupts α-crystallin chaperone activity, which is crucial for maintenance of lens transparency (22, 23). Therefore, L-carnitine may affect lens transparency by acting directly on α-crystallin. In fact, L-carnitine can contribute to the acetylation of the protein--a process that seems to protect crystallin from molecular modifications that decrease its chaperone activity (33). The relatively large pool of lens carnitine acts as a buffering system that stabilizes the ratio of acetylated to free coenzyme A (51). Free carnitine can be acetylated whenever a mismatch occurs between the fluxes through pyruvate dehydrogenase and the tricarboxylic acid cycle, which occurs in cell oxidative stress (52–54). Indeed, we found that acetyl-L-carnitine was increased in lenses treated with L-carnitine and exposed to H_2O_2. The increase of acetyl-L-carnitine could favor enzymatic or nonenzymatic crystallin acetylation (23, 35).

Both α- and β-crystallins are N-terminally acetylated. Using screening spot-blot analysis combined with mass spectrometry, Takemoto et al. provided evidence that the N-acetylated-terminal methionine of α-A-crystallin can be oxidized to methionine sulfoxide in vivo (55). Oxidation of the N-terminal methionine, which is exposed on the outside of the polypeptide, can negatively affect the function of the protein. In addition to NH_3-terminal acetylation, the ε-amino groups of lysine (Lys) residues are subject to acetylation. All seven Lys residues of bovine α-A-crystallin react with aspirin, the extent of acetylation varying from 10% for Lys 88, the least reactive, to 60% for Lys 166, the most reactive (56, 57). Aspirin, a putative anticataract agent, inhibits both glycation and carbamoylation as well as aggregation of lens proteins, presumably through acetylation of Lys residues (58–63). Recently, it has been shown that acetyl-L-carnitine inhibits glycation of α-crystallin--to a greater extent than do other crystallins--through acetylation of the potential glycation sites (35). Glycation seems to be more harmful than acetylation because only glycation products are involved in protein cross-linking and in a significant decrease of the α-crystallin chaperone activity (35, 64).

It remains to be established whether or not L-carnitine is involved in maintaining correct crystallin folding in cells exposed to oxidative stress. We have proposed that carnitine, as a member of the methylamine family, be viewed as an organic compatible solute that stabilizes

protein, thus mimicking the ions of the Hofmeister series (34). In the Hofmeister series, the degree of methylation of the nitrogen atoms of substituted ammonium ions enhances their ability to stabilize macromolecules. Thus, in the case of organic methylamine, it is not inconceivable that fully methylated compounds such as carnitine could be one of the most stabilizing factors.

In this study, we report experimental evidence that an increase of lens protein cross-links induced by TGase parallels both the oxidative stress and the decrease in α-crystallin chaperone activity in lens organ culture. It is not known why lens proteins sustain a greater degree of TGase-mediated modifications consequent to oxidative stress. Groenen et al. (32) speculated that oxidation induces conformational changes in lens crystallins that unmask specific domains, thereby increasing their susceptibility to act as TGase substrates. Alternatively, the increased susceptibility to TGase might be due to a decrease in α-crystallin chaperone activity to levels at which it can no longer prevent aggregation of lens crystallins. Changes in the stability of lens proteins could be crucial in regulating the posttranslational modifications that occur during cataract development. A close analogy for some of the changes that may occur in lens protein aggregates can be drawn from the stabilization of fibrin clots by activated factor XIII, a member of the TGase family of enzymes (64–66). The reversible self-assembly of fibrin molecules into a clot greatly accelerates the rate of the enzyme-catalyzed cross-linking reaction (66).

It also remains to be established when and how TGase is activated in the lens (67). Like liver TGase, the lens enzyme has a dual role: to function as a GTP-binding protein in signal transduction and to mediate lens remodeling reactions (68). The concentration of GTP would affect the cross-linking potential of the lens enzyme, and a significant drop in the concentration of the nucleoside triphosphate, which occurs in cataract formation, would significantly favor TGase expression. L-carnitine depletion could lead to decreased energy production from mitochondrial oxidation and consequently to reduced ATP and GTP synthesis.

A novel explanation of the protective effect of carnitine on TGase-mediated cross-link formation could be related to the capacity of this molecule to favor acetylation of target proteins. As occurs during glycation, lysine residues participate in the cross-linking reaction mediated by TGase, whereas the acetylated form is no longer a substrate of the enzyme. It is likely that a combination of two or more of the previously mentioned mechanisms underlies the protective effect exerted by L-carnitine on lens transparency.

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Table 1

Changes of several biochemical parameters in control lenses and in lenses treated and not with L-carnitine and exposed to H_2O_2.

	Lens wet weight (mg)	Water insoluble protein (%)	Glutathione (μmoles/g w-w)	Free carnitine (nmoles/g w-w)	Acetyl-L-carnitine (nmoles/g w-w)	Lactate dehydrogenase (units/ml conditioned media)	Total carnitine (nmoles/g w-w)
Controls	25 ± 0.1	5 ± 1.1	4.87 ± 0.23	156 ± 3	29 ± 1	ND	187 ± 5
H_2O_2 (500 μM)	47 ± 0.2*	41 ± 1.9*	2.44 ± 0.69*	27 ± 2*	6 ± 1*	53 ± 6	36 ± 3*
L - CAR (300 μM) + H_2O_2 (500 μM)	27 ± 0.9	5 ± 2.0	2.71 ± 0.73*	151 ± 2	37 ± 2**	ND	189 ± 2

*p< 0.001 **p< 0.005
ND: not detectable

Table 2

Effects of α-crystallins from control and H_2O_2 ± L-CAR treated lenses on gel formation by intermediate filaments as monitored by a falling ball viscometry assay

	GFAP gel formation as indicated by the ball position in the viscosimetry
Controls	TOP
H_2O_2 (500 μM)	BOTTOM
L - CAR (300 μM) + H_2O_2 (500 μM)	TOP

Fig. 1

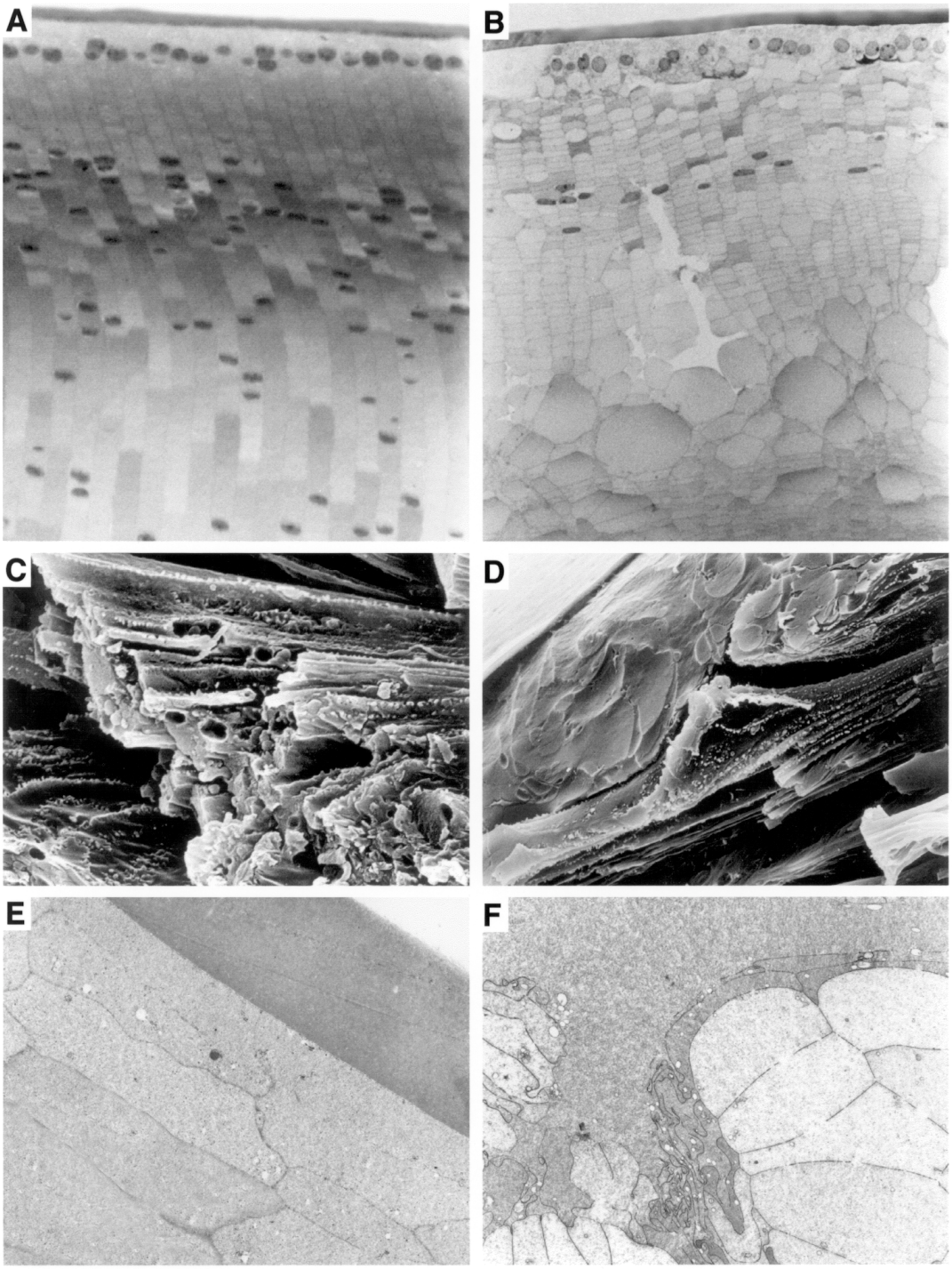

Figure 1. Microscopic examination of lenses treated with L-carnitine and H_2O_2 (A, C, E) or with H_2O_2 alone (B, D, F). Optical microscopy. **(A)** The lens treated with L-carnitine and H_2O_2 retains a regular appearance and is covered by a thin epithelial cell layer and a normal, thick lens capsule (magnification: 250×). **(B)** The lens treated with H_2O_2 alone is disrupted; note the areas of complete fiber degeneration and liquefaction and the swelling and ballooning of the fibers (magnification: 250×). Scanning electron microscopy. **(C)** Lens morphology and fiber order are maintained in a lens treated with L-carnitine and H_2O_2 (original magnification: 1000×). **(D)** Disrupted areas and liquefaction are clearly evident in a H_2O_2-treated lens (original magnification: 1000×). Transmission electron microscopy. **(E)** Again, the lens treated with L-carnitine and H_2O_2 has a normal morphology (original magnification: 4000×). **(F)** The ballooning of fibers, disrupted areas, etc., are confirmed in a lens treated with H_2O_2 alone (original magnification: 4000×).

Fig. 2

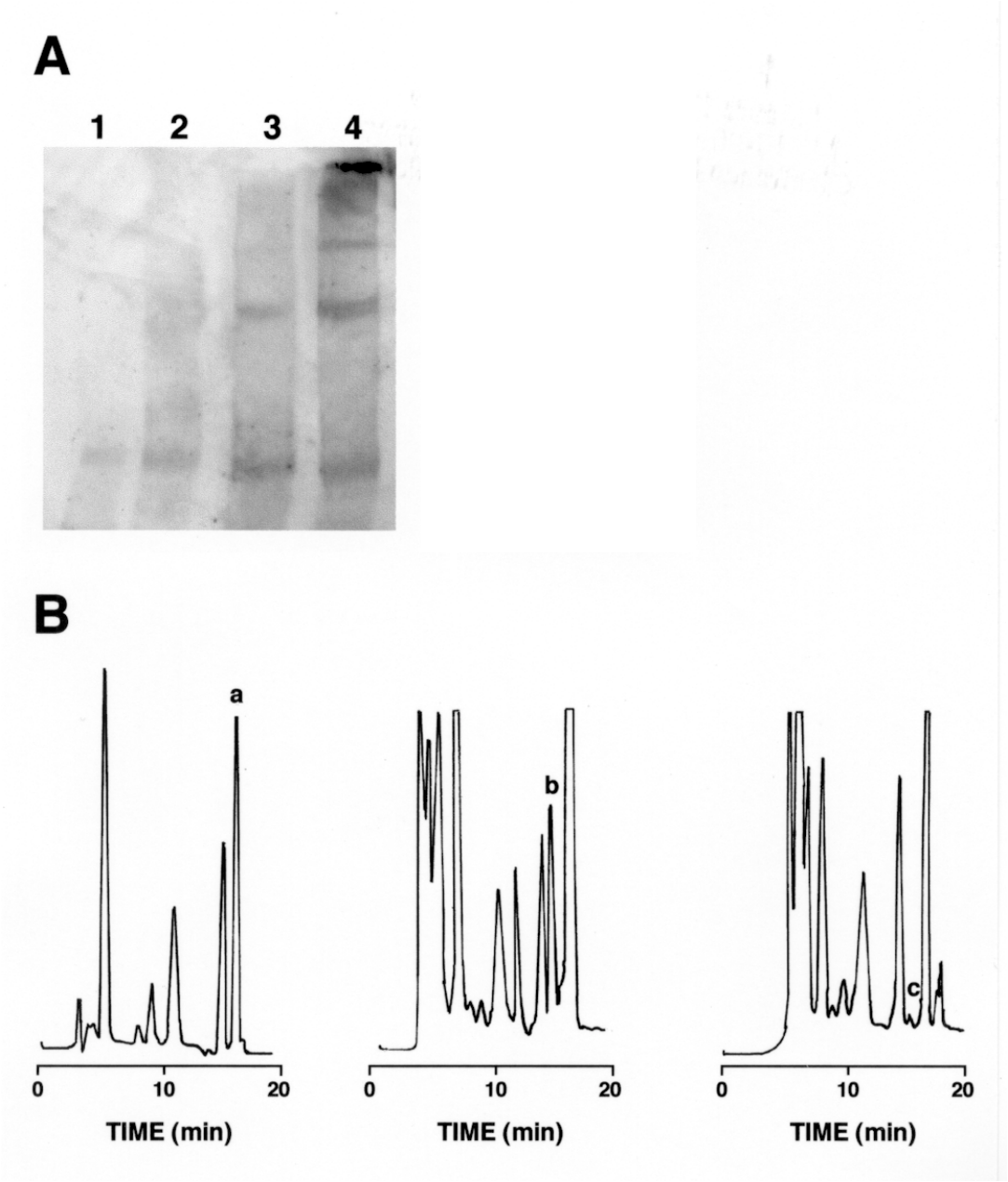

Figure 2. Cross-linked high molecular weight proteins in water-insoluble lens fractions and chromatographic patterns of the phenyl-thiocarbamyl (PTC) derivate of the ε-(γ-glutamy)lysine isopeptide from three groups of lenses examined. (A) Lane 1: Western blot of water-soluble lens fractions. Lane 2: water-insoluble control specimens. Lane 3: lenses treated with both L-carnitine and H_2O_2. Lane 4: lenses treated with H_2O_2 alone. **(B)** Left: chromatographic profile of exogenous pure isopeptide (5 pmol) added to exhaustive enzyme digestion of noncross-linked, target protein (albumin); the PTC-isopeptide elutes at 17.04 min (a). Middle: chromatographic profile of the water-insoluble lens protein fraction after exhaustive enzyme digestion of samples treated with H_2O_2 alone. Right: chromatographic profile of the water-insoluble lens protein fraction after exhaustive enzyme digestion of samples treated with L-carnitine and H_2O_2.

Fig. 3

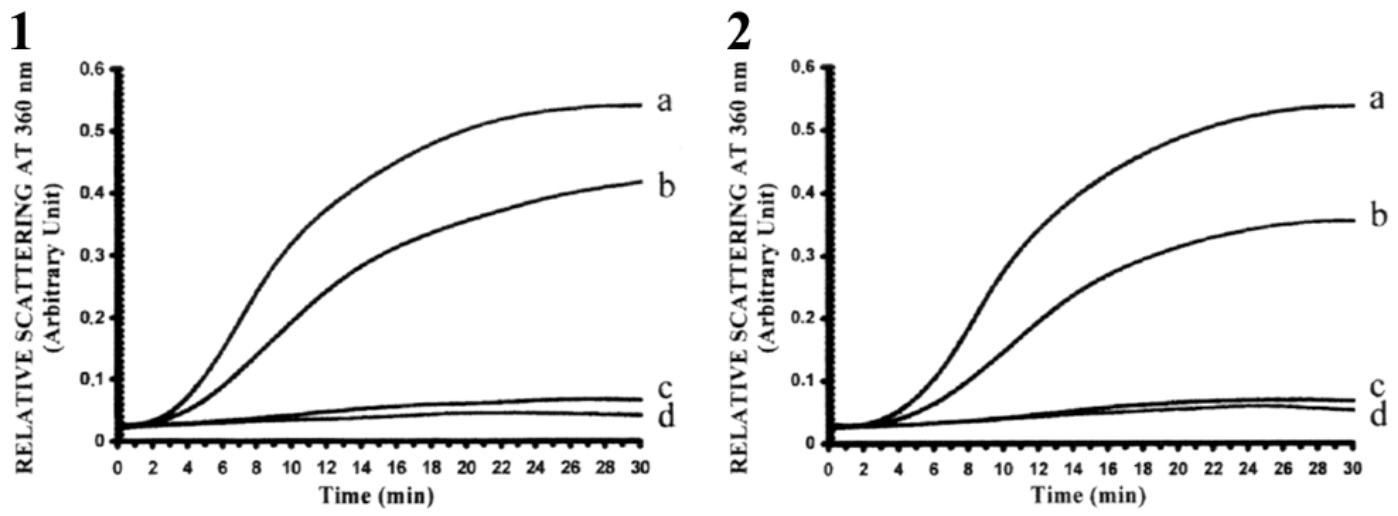

Figure 3. Chaperone-like activity of the water-soluble α-crystallin fraction obtained from control lenses and from lenses treated with H_2O_2 in the presence or absence of L-carnitine determined by heating assays and recorded in a spectrophotometer at 360 nm absorbance. Heating assays were performed with 200 µg β_L-crystallin, and 100 µg **(1)** or 200 µg **(2)** α-crystallin. Curve a: substrate protein alone; curve b: substrate protein and α-crystallin obtained from lenses treated with H_2O_2 alone; curve c: substrate protein and α-crystallin obtained from lenses treated with L-carnitine and H_2O_2; curve d: substrate protein and α-crystallin obtained from control lenses.