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LABAROTARY STUDY

Decreased chaperone activity of α-crystallin in selenite cataract may result from selenite-induced aggregation

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Abstract

Purpose **To investigate the role of chaperone activity of α-crystallin in selenite-induced cataract formation.**

Methods **Selenite cataract was induced in Sprague–Dawley rats by five subcutaneous injections of sodium selenite over a 20-day period starting at 8–10 days postpartum. α-Crystallin was separated from the rat lenses by size-exclusion chromatography. Bovine α_L-crystallin and β_L-crystallin were isolated for studies *in vitro*, and for the chaperone assays. The protective effects of both α_H- and α_L-crystallin were measured spectrophotometrically in four different assay procedures including the thermally induced aggregation of catalase and β_L-crystallin, and the fructation- and heat-induced inactivation of catalase. The bovine α_L-crystallin was incubated with different concentrations of sodium selenite for 72 h and then its chaperone activity against heat-induced β_L-crystallin aggregation was assayed. The aggregation of selenite-treated α_L-crystallin was analysed by molecular sieve high-performance liquid chromatography (HPLC).**

Results **The protection of α_H-crystallin was less than that of α_L-crystallin in both normal and cataractous lenses. The chaperone activities of both α_H- and α_L-crystallin in selenite cataract were decreased compared with normal lenses. The protection provided by both α_H-crystallin and α_L-crystallin against the thermal aggregation of catalase was much greater than their protection against thermally and chemically induced inactivation. HPLC analysis demonstrated aggregation of α-crystallin by sodium selenite after 24 h incubation in a dose-dependent fashion.**

Conclusion **The chaperone activity of α-crystallin presented parallel patterns of activity with different methods, further supporting the view that the different assays measure essentially the same property. The decreased chaperone activity of α-crystallin in selenite cataract may result from selenite-induced aggregation.**

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Keywords: molecular chaperone; α-crystallin; selenite-induced cataract; catalase; aggregation

Introduction

The selenite-induced cataract produced in young rats has been used extensively as a model for nuclear cataract. Its reliability and reproducibility result in a good rodent model for testing potential anticataract drugs.[1–6] Since Ostadalova *et al*[7] first introduced this model, numerous experiments have shown that both a single[8–11] or repeated subcutaneous injections of smaller doses of selenite,[12] and oral selenite administration[13] are cataractogenic. Selenite nuclear cataract appears within 3–5 days after a single injection of an overdose of sodium selenite. However, dense cortical cataract was also reported at 15–30 days after a single subcutaneous injection in addition to previously characterized nuclear changes, although it cleared subsequently.[10]

The major biochemical findings accompanying the development of selenite cataract include altered epithelial metabolism, calcium accumulation, calpain-induced proteolysis, crystallin precipitation, phase transition, and cytoskeletal loss.[2,14,15] The

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β-crystallin is degraded in the selenite cataract.[16,17] Limited proteolysis of crystallins, especially β-crystallin polypeptides, leads to loss of the N-terminal extensions on β-crystallins, abnormal interaction of crystallins, insolubilisation of proteolyzed β- and α-crystallins, coprecipitation of γ-crystallins, and loss of cytoskeletal proteins and soluble proteins.[18,19]

Mainly β-crystallins are precipitated, but there is concurrent calpain-induced proteolysis of α-crystallins, which leads to a marked reduction in α-crystallin chaperone activity in selenite nuclear cataract[20] and Shumiya cataract[21] in rat. α-Crystallin has chaperone-like properties, being capable of binding to unfolded or denatured proteins and suppressing nonspecific aggregation.[22] The chaperone function of α-crystallin helps to prevent the formation of large light-scattering aggregates, inactivation of enzymes, and possibly cataract.[23,24]

Different assays for chaperone function of α-crystallin have been developed including heat- and UV-induced protein aggregation, sugar, and steroid inactivation of enzymes.[23,25] Kelley *et al*[20] showed that the chaperone activity of α-crystallin from the nucleus of rat lenses was diminished in selenite cataract, but their only chaperone assay was a β_L-crystallin aggregation assay at 64°C, far from physiological temperatures. Assays at physiological temperature are to be preferred. Some studies of mutant α-crystallins have led to confusion because they showed no chaperone function at elevated temperatures simply because the proteins were less stable than wild type.[26]

Catalase is a crucial antioxidative enzyme in the normal lens.[27] Its activity decreased with ageing and in human cataract.[28,29] The evaluation of chaperone activity of α-crystallin using catalase assays has been established by our group including thermal aggregation of catalase, inactivation of catalase by fructose,[30] steroid,[31] and thermal stress.[32] α-Crystallin can protect catalase activity against thermal or glycation-induced inactivation and aggregation on the denaturation pathway, but it shows different abilities to promote functional refolding by preventing proteins from misfolding or entering kinetic traps on the renaturation pathway.[31,32]

Incubation of cultured rat lenses with 0.2 mM selenite for 24 h, resulted in an increase in selenium content and lens opacity.[33] However, the exact role of the chaperone function provided by α-crystallin in the pathogenicity of selenite cataract remains to be elucidated.

The purpose of this study was to investigate the molecular chaperone activity of α-crystallin in normal and selenite-induced cataractous lenses using four different assays including thermal aggregation assays of catalase and of β_L-crystallin, and catalase inactivation assays by glycation and by heat. The chaperone activities of both HMW and α_L-crystallin from completely cataractous lenses were also measured compared with normal lenses. The cataract included both cortical and nuclear regions in contrast with most previous studies, which have been on pure nuclear cataract. The effect of sodium selenite on chaperone activity of α-crystallin was further investigated *in vitro* and its possible aggregation was assessed by high-performance liquid chromatography HPLC. The different assays demonstrated a similar pattern of results. The observations *in vitro* reinforce evidence that chaperone function may contribute to the development of aggregated proteins in the selenite cataract formation, and showed that selenite can directly cause aggregation of α-crystallin and loss of its chaperone function.

Materials and methods

Materials

The catalase from bovine liver (EC 1.11.1.6), fructose, bovine serum albumin, lysozyme, and SDS were purchased from Sigma Chemical Co (Beijing, China). Sephacryl S-300HR was from Pharmacia Ltd (Beijing, China). Sodium selenite was from Merck (Lutterworth, UK). BioSep-SEC-S4000 was obtained from Phenomenex (Macclesfield, UK).

Animals

Seven litters of Sprague–Dawley rats at 8–10 days postpartum were provided by Animal Laboratories (Xi'an, China), and were housed in individual polypropylene breeding cages under a day/night cycle of 12 h, at 25°C room temperature, and with both the male and female parents present throughout weaning. The rats received laboratory chow and distilled water *ad libitum*. The rat experiment conformed to accepted principles of animal maintenance and care.

Half the pups of each litter were daily given subcutaneous injections of 20 μmol sodium selenite/kg body weight in 0.9% NaCl in the scruff of the neck starting at 8–10 days postpartum and served as the cataract group, while the remaining pups of each litter were injected with saline over 5 days and served as the control.

When the eyelids opened at 13–15 days postpartum, eyes were dilated with 0.5% atropine sulphate (Atropine, Alcon) and examined with a slit lamp (Zeiss). After 20 days of injections, 74 both cortical and nuclear cataractous and 80 clear lenses were removed immediately after decapitation of the rats.

Isolation of crystallins

The α-crystallin was isolated from rat lens by size-exclusion gel chromatography on Sephacryl S-300HR as described by Slingsby and Bateman,[34] and Derham and Harding.[35] Bovine α_L-crystallin and β_L-crystallin were separated by the same method. Decapsulated lenses were weighed and homogenised in seven times their mass of 0.05 M sodium phosphate buffer, pH 6.7. The homogenates were centrifuged at 22 440 *g* for 40 min at 4°C to separate the soluble and insoluble lens proteins. The supernatants were loaded onto the chromatography column ($1.6 \times 100\,cm^2$) and proteins eluted using the same buffer at a flow rate of 18 ml/h. The fractions corresponding to each standard peak as α-high and α-low in rats or β-low in bovine lenses were pooled separately, dialysed against at least three changes of distilled water over 24 h at 4°C. The pooled fractions were separately freeze-dried, and stored at −20°C until required. The purity of α-crystallin was confirmed by SDS-PAGE electrophoresis.

Incubation of bovine α_L-crystallin with sodium selenite

Bovine α_L-crystallin, 30 mg, was dissolved in 15 ml sodium selenite, over a concentration range of 0, 0.4, 4, 10, 50, and 100 mM. The solution was then filtered through a sterilised 0.2 μm pore-sized sterilized Millipore filter into a sterilized 30 ml glass vial with silicone bung and incubated at 37°C in a shaking water bath. At time zero, 24, and 72 h, 3 ml samples were individually removed from each of the solutions by syringe and then were dialysed in microdialysis tubing with a minimum of three changes of 5 l of distilled water over 24 h. After freeze-drying, the chaperone activity of α-crystallin was determined using the β_L-crystallin aggregation assay and further analysed by HPLC.

HPLC analysis

HPLC was carried out using a Gilson 714 HPLC equipped with a BioSep-SEC-S4000 ($300 \times 7.8\,mm^2$) size-exclusion column. Protein samples of 0.5 ml were taken from incubation experiments at 24 h. They were then centrifuged at 2800 *g* for 5 min before being injected on to the column (up to 50 μl). All analyses were performed at ambient temperature and eluted with 0.1 M sodium phosphate buffer (pH 6.9) at a flow rate of 1 ml/min. Protein elution was monitored at 280 nm.

Aggregation assays

The heat aggregation assays were based on those described by Derham and Harding[35,36] and Hook and Harding[31] with slight modifications. The ratio of 1 : 2.5 (w/w) corresponding to 100 μg of α-crystallin to 250 μg target proteins (β_L-crystallin and catalase) was used for chaperone assays. Lysozyme and bovine serum albumin were examined in every experiment as controls substituting for the α-crystallin. The scattering resulting from aggregation at 60°C was measured for 60 min at 360 nm in a Kontron 930 spectrophotometer as an index of turbidity. All assays were repeated in triplicate. Chaperone activity was determined as a percentage of protection relative to the target protein control after 60 min.

Sugar-induced inactivation assay

The enzyme inactivation assay by sugar was based on that described by Hook and Harding.[31,32] α-Crystallin was present at 40 μg/ml in a solution containing catalase (525 IU, 25 μg/ml) and 5 mM fructose. Catalase activity was assayed at 2-day intervals over 6 days incubation in a shaking water at 37°C.

Inactivation assay by heat

Catalase (525 IU, 25 μg/ml) was incubated at 60°C with and without 40 μg/ml α-crystallin, bovine serum albumin, or lysozyme for 40 min in a final volume of 1 ml. Activity was assayed at 10 min intervals. Chaperone ability was represented as a percentage of activity remaining at the end of incubation compared to the corresponding α-crystallin incubation.

Catalase assay

The assay of catalase activity was performed at 37°C as described previously by monitoring the decreased absorbance at 240 nm resulting from the decomposition of hydrogen peroxide for 1 min in a reaction mixture (3 ml final volume) containing 2.98 ml of solution from diluted 30% (w/v) H_2O_2 and 20 μl incubation solution in 50 mM sodium phosphate buffer, pH 7.[37] All assays were carried out in triplicate. Activity is expressed relative to the control activity at each respective time interval, set at 100%.

SDS-PAGE

SDS-polyacrylamide gel electrophoresis of proteins was based on Laemmli's system under reducing conditions[38] by using Electrophoresis Units (Bio-RAD) on composite gels consisting of a 5% stacking gel and a 12.5% resolving gel. The gel was stained by Coomassie blue R-250.

Analysis

The statistical significance of differences were assessed with Student's *t*-test using SPSS (Version 10.0) software. Where error bars are displayed on graphs, these represent standard deviations of three individual experiment from each group.

Results

Incidence of cataract

Six days after injection, the incidence of nuclear cataract was 90% (70/78), and cortical plus nuclear cataract was 6% (5/78). The frequency of complete opacification of the lenses had risen to 95% (74/78) 20 days after injections started (Table 1).

In a preliminary experiment, the ineffectiveness of selenite after 15–20 days postpartum was found in 10 rats even though an increased amount of sodium selenite (60 mol/kg body weight) was injected (results not shown).

Isolation of lens crystallins

The elution profile from clear and cataractous lenses of soluble proteins showed an increase of the α_H-crystallin peak and a slight decrease of both β_H- and β_L-crystallin peaks in selenite cataract lenses, while the γ-fraction appeared to be unaffected (Figure 1). The fractions of α_H- and α_L-crystallin contained approximately 39 and 61% of total α-crystallin, respectively, in clear lenses as assessed by the elution profile on Sephacryl S-300HR. However, the percentage of α_H- and α_L-crystallin changed to 49 and 51% of total α-crystallin in selenite cataract lenses. Both α_H- and α_L-crystallin from control and cataractous lenses were further analysed by SDS-PAGE (Figure 2). The α_H-crystallin from cataractous lenses contained a few new polypeptides below 20 kDa. New 25 and 23 kDa bands were also present in α_H- and α_L-crystallin. These changes were previously reported in nucleus of the pure nuclear cataract model.[16]

Chaperone activity of α-crystallin assessed using four different assays

Protection by α-crystallin against heat-induced aggregation of catalase and β_L-crystallin, and fructation- and heat-induced inactivation of catalase are shown (Figure 3a–d). The pattern of the protective effect in both

Table 1 The incidence of cataract induced by five subcutaneous injections of sodium selenite in young Sprague–Dawley rats over a 20-day period starting at 8–10 days postpartum

Group	*Number (rats)*	*Cataract*	*Clear or cataractous lenses after injection*					
			3 days	*6 days*	*9 days*	*12 days*	*15 days*	*20 days*
C	40	–	80	80	80	80	80	80
S	39	–	22	3	0	0	0	0
		+	56	70	36	20	16	4
		*	0	5	42	58	62	74

C: control; S: selenite-treated; –: clear lens; +: pure nuclear cataract lens.
*: Combined cortical and nuclear cataract lens.

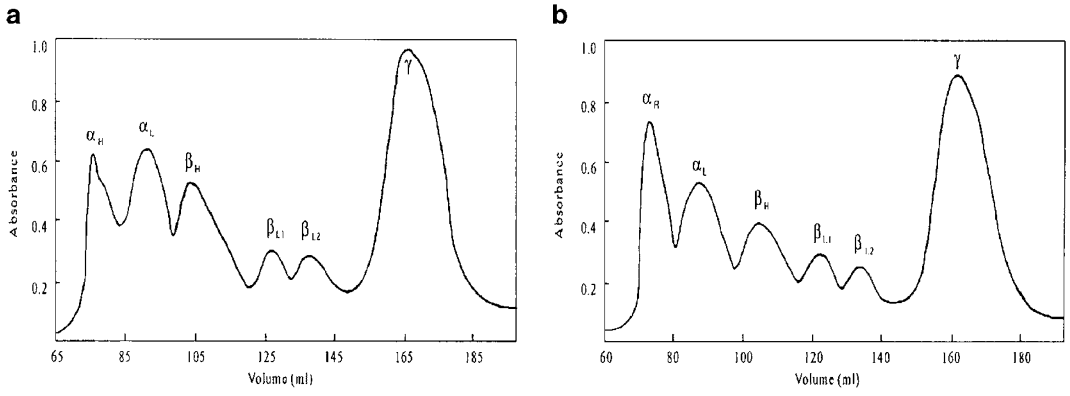

Figure 1 Elution profiles from size-exclusion chromatography using Sephacryl S-300HR of water-soluble proteins from clear and selenite-induced cataract lens in rats. (a) Clear lens. (b) Selenite-induced cataract lens. The α_H, α_L, β_H, β_L, and γ represent the corresponding crystallin elution peaks.

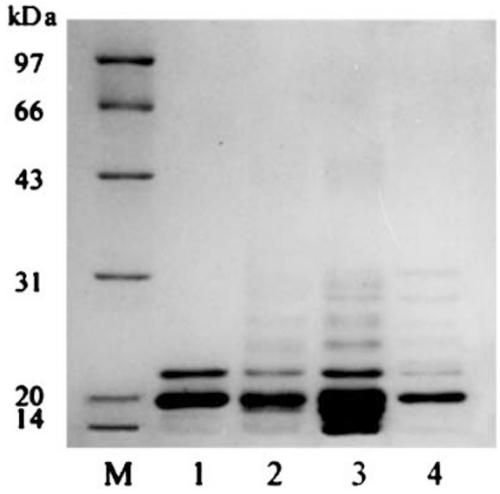

Figure 2 SDS-PAGE analysis of α-high and α-low crystallin preparations from clear and selenite-induced cataract lenses in rats. Lane M, molecular weight standards labelled in the margin in kilo Daltons; lanes 1 and 2, α-low and α-high from clear lens; lanes 3 and 4, α-high and α-low from selenite-induced cataract lens, respectively. Note the appearance of proteolytic fragments below the 20 kDa band in lane 3.

α_H- and α_L-crystallin is very similar. The protection of α_H-crystallin from normal and cataractous lenses was approximately 28.7% ($P<0.001$) and 23.7% ($P<0.001$) less than that of α_L-crystallin with the catalase aggregation assay (Figure 3a), 10.9% ($P=0.028$) and 13.4% ($P=0.022$) with the β_L-crystallin aggregation assay (Figure 3b), 8% ($P=0.032$) and 5.7% ($P=0.142$) with the fructation inactivation assay (Figure 3c), 5% ($P=0.146$) and 2.1% ($P=0.263$) with the heat inactivation assay (Figure 3d), respectively.

The chaperone activities of α_H- and α_L-crystallin in selenite cataract were diminished approximately 14.7% ($P<0.001$) and 19.7% ($P<0.001$) according to the catalase aggregation assay (Figure 3a), 8.5% ($P=0.001$) and 6% ($P=0.047$) by the β_L-crystallin aggregation assay (Figure 3b), 14% ($P<0.001$) and 16.3% ($P<0.001$) by the glycation inactivation assay (Figure 3c), and 7% ($P=0.008$) and 10% ($P=0.003$) by thermal inactivation assay (Figure 3d) compared with normal lenses, respectively. α-Crystallin was more effective in preventing the thermal aggregation of catalase than of β_L-crystallin. The control proteins, lysozyme and serum albumin, displayed no such protective ability when substituted for α-crystallin (results not shown).

Fructose inactivated catalase in a progressive manner during 6 days incubation. In the presence of 5 mM fructose, catalase activity fell to 15 and 6% after 4 and 6 days, respectively; but with α-crystallin from clear and cataractous lenses, activity remained at 50–70% and fell to 35–60% after the same time periods, respectively. Clearly, α-crystallin could partly protect against inactivation by 5 mM fructose for a limited period of time. There was a subunit protection ratio of 5 : 1 (α-crystallin : catalase) or each molecule of α-crystallin protected two molecules of catalase, assuming the α-crystallin exits as a complex of 800 kDa.

Protection by α-crystallin against thermal inactivation of catalase appeared to be conferring 13–18 and 5–9% protection in clear and cataractous lenses, respectively, after 40 min (Figure 3d). α-Crystallin conferred statistically significant, but not full, protection against catalase thermal inactivation at 60°C in the same protection ratio as above. It showed a much less efficient protection against the thermally induced inactivation of catalase at 60°C than it did against fructose-induced inactivation at 37°C, emphasising the importance of chaperone assays at physiological temperatures.

Chaperone activity of α-crystallin with selenite-treated and HPLC analysis

The effect of sodium selenite on chaperone activity of bovine α_L-crystallin is shown in Figure 4. The chaperone activity was significantly decreased after 24 h incubation with 10, 50, and 100 mM sodium selenite in comparison with the control group. There was no further falling in chaperone activity after 72 h incubation (results not shown).

α_L-Crystallin was subject to HPLC analysis after treatment with various selenite concentrations (Figure 5). Curve a was the gel filtration profile of normal α_L-crystallin without selenite-treatment as a reference; no aggregate peak was observed. Incubation at 37°C did not cause aggregation of normal α_L-crystallin (Figure 5a, b). There was an unambiguous formation of aggregates after incubation with selenite for 24 h, the amount of which gradually increased as shown from curves c to f. The peak of α_L-crystallin decreased and shifted to the high molecular weight at least up to 50 mM selenite. Equivalent amounts of α-crystallin were noted in curves c–f, suggesting that the aggregated form consists mostly of α-crystallin itself. These data are consistent with the reduction of its chaperone activity, indicating that selenite induced the aggregation of α-crystallin in a dose-dependent manner. This in turn showed that the decreased chaperone activity in selenite cataract may contribute to aggregation in cataract formation. The maximum effect on both chaperone activity (Figure 4) and aggregation (Figure 5) was at 50 mM sodium selenite.

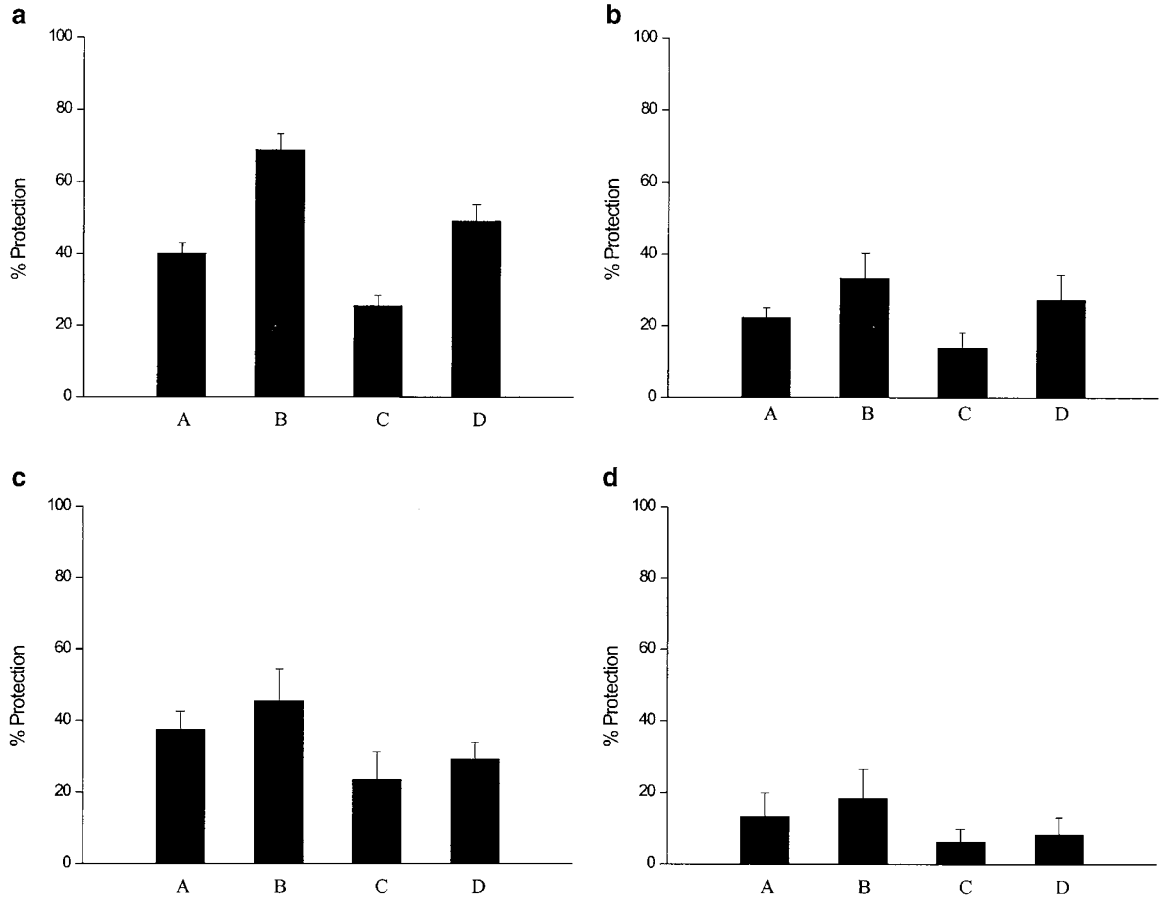

Figure 3 Chaperone activity of rat α_H- and α_L-crystallins from clear and selenite-induced cataract lens. Chaperone activity is represented as a percentage of protection relative to the target proteins in aggregation assays and as a percentage of activity remaining in inactivation assays. Each data point is represented as the mean $\pm$SD of three readings in each segment. A, α_H-crystallin from clear lens; B, α_L-crystallin from clear lens; C, α_H-crystallin from selenite-induced cataract lens; D, α_L-crystallin from selenite-induced cataract lens. (a) Protection against heat-induced aggregation of catalase (250 μg) at 60°C by α_H- and α_L-crystallins (100 μg) from clear and selenite-induced cataract lens in rats. Chaperone activity was represented as a percentage protection relative to the catalase control after 60 min. (b) Protection against heat-induced aggregation of β_L-crystallin (250 μg) at 60°C by α_H- and α_L-crystallins (100 μg) from clear and selenite-induced cataract lenses in rats. Chaperone activity was represented as a percentage protection relative to the β_L-crystallin control after 60 min. (c) Protection of enzyme activity following fructation (5 mM) of catalase (25 μg/ml) at 37°C by α_H- and α_L-crystallins (40 μg/ml) from clear and selenite-induced cataract lens in rats. Protection was obtained as a percentage of activity remaining compared to an enzyme only at 6 days incubation. (d) Protection of enzyme activity following thermal inactivation of catalase (25 μg/ml) at 60°C by α_H and α_L-crystallins (40 μg/ml) from clear and selenite-induced cataract lenses in rats. Protection was obtained as a percentage of activity remaining compared to a control with enzyme alone after 40 min incubation.

Discussion

Selenite is cataractogenic only when administered to young rats before completion of the critical maturation period of the lens (approximately 16 days of age).[2] The timing of injection is very critical possibly because of the sharply decreasing uptake of selenium by the lens.[39] Severe cortical cataract, in addition to the nuclear cataract previously characterised, develops in lenses approximately 15–30 days after a single injection of selenite. By 25–35 days, 77% of the selenite-injected rats developed cortical cataract although they subsequently became clear after 4 months.[10] Our results showed that both cortical and nuclear cataracts were present in 94% of rats after five subcutaneous injections of sodium selenite over a 20-day period, and in 70% of the rats dense nuclear opacity appeared by 3 days after the last injection. It is suggested that, given the overdose of selenite in Sprague–Dawley rats, it is possible to shorten the timing of the appearance of the total cataract formation.

The present results showed an increase of the α_H-crystallin and decrease of the α_L-crystallin in cataractous lenses compared with clear lenses. Both

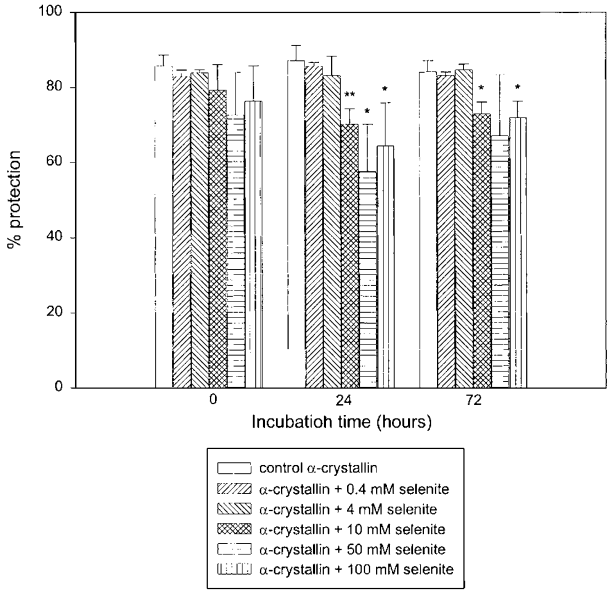

Figure 4 Effect of sodium selenite on the chaperone activity of bovine α_L-crystallin as assessed by the β_L-crystallin aggregation assay. Protection was obtained as a percentage of protection relative to the β_L-crystallin control at 0, 24, and 72 h incubation. Each point represents the mean of three measurements. $^*P<0.05$ and $^{**}P<0.01$ are the levels of significance. Data are presented as mean $\pm$ SD.

α_H- and α_L-crystallin preparations from the completely cataractous lenses contained a few new bands, which corresponded in size to several β_H- and β_L polypeptides. This is consistent with the earlier report on nuclear cataract after a single injection of selenite in 10-day-old rats,[16] which indicated that proteolysis had resulted in a decrease in β_H- and β_L-crystallin in the nucleus, and loss of many polypeptides from the soluble, insoluble, and intrinsic membrane fractions. Isolated cortical crystallins showed no significant changes in polypeptide composition following selenite cataract formation,[10,20] which is surprising given that calpain activity is found in lens cortex, but not in nucleus.[40] Truncated proteins have been identified in lens, therefore proteolysis of lens proteins may play a role in cataract formation.[41] Activation of the cysteine protease calpain II may be responsible for decreased chaperone activity in selenite cataract.

Different assays of chaperone function at physiological and at elevated temperatures exhibited similar patterns of protection in the present study. However, the protective effect against inactivation of catalase by glycation at 37°C was much greater than that by heat at 60°C. Glycation probably inactivates catalase by conformational changes of the enzyme and the protective effect of α-crystallin is probably by stabilising the native structure. The thermally induced conformational change to the target proteins leads to noncovalent binding to α-crystallin, which binds hydrophobic regions revealed by unfolding target proteins with the generation of soluble complexes. The cumulative evidence of the protection of α-crystallin against inactivation of all enzymes investigated and thermal aggregation of enzymes and other proteins suggests the lack of substrate specificity of α-crystallin.[23,25] Previous experiments in ageing and human cataract indicated that post-translational modification may be responsible for decreases of α-crystallin chaperone activity, and it shows a similar pattern by aggregation and inactivation of malate dehyrogenase assays.[35,36] Sorbitol dehydrogenase was significantly protected from both thermally induced inactivation and aggregation by bovine lens α-crystallin.[42] Our results further support the view that the different assays are measuring essentially the same property, but present quantitatively different protective effects with different methods.

The α_H-crystallin showed compromised protection against aggregation and inactivation of proteins in normal and cataractous lenses as reported previously.[35,43,44] α_H-Crystallin has been considered to be an intermediate between the soluble and insoluble fractions, which contained more bound β- and γ-crystallins than α_L-crystallin. A decrease in α_L-crystallin with an increase in α_H-crystallin in selenite lens would lead to a decrease in active α-crystallin to provide protection. This may result in reduced suppression of nonspecific aggregation and inactivation of enzymes in cataractous lens. This is in addition to the decreased function of both fractions in the selenite cataract lenses.

In the *in vitro* study, chaperone activity of selenite-treated bovine α-crystallin decreased significantly after 24 h in a dose-dependent manner. The concentration of sodium selenite used *in vitro* is higher than that expected *in vivo* in the rat, but there is more time for modification by selenite to occur *in vivo*. Chaperone activity of α-crystallin *in vivo* may differ from that determined *in vitro*.[45]

HPLC analysis showed that the formation of high-molecular-weight aggregates (HMWA) of α-crystallin increased with the elevated concentration of selenite, indicating that loss of chaperone activity is concomitant with the formation of HMWA. The decreased chaperone activity of α-crystallin resulting from selenite-induced aggregation, and the degradation of lens proteins induced by m-calpain in rat, may combine to promote the aggregation of lens proteins, which could lead eventually to light scatter and cataract.

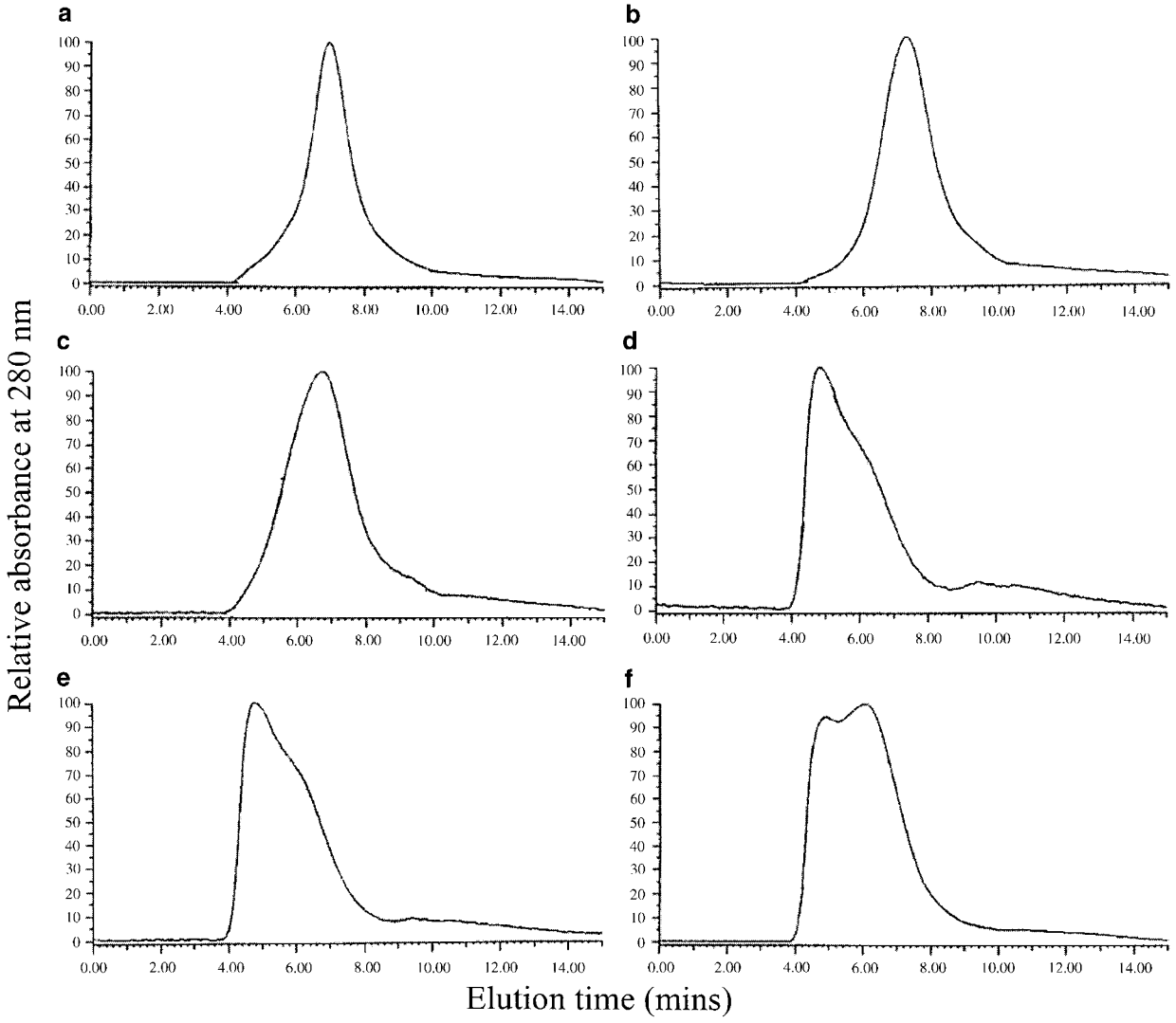

Figure 5 HPLC gel filtration profiles of bovine α_L-crystallin after being incubated with various concentrations of sodium selenite for 24 h. Each protein was at a concentration of 0.3 mg/ml. Sodium selenite concentration: (a) normal α_L-crystallin without incubation; (b) normal α_L-crystallin without selenite treatment after 24 h incubation; (c) 4 mM; (d) 10 mM; (e) 50 mM; (f) 100 mM sodium selenite.

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