Journal of Protein Chemistry, Vol. 16, No. 4, 1997

α-Crystallin Acting as a Molecular Chaperonin Against Photodamage by UV Irradiation

Jiahn-Shing Lee,[1] Jiahn-Haur Liao,[2] Shih-Hsiung Wu,[2,3] and Shyh-Horng Chiou[2-4]

Received December 3, 1996

α-Crystallin, a major protein of the eye lens, is known to have chaperone activity in preventing heat-induced aggregation of enzymes and other crystallins. In this study, we investigate the ability of α-crystallin to inhibit UV-light-induced aggregation of other lens proteins and the effect of exposure of α-crystallin to UV irradiation on its chaperone activity. The chaperone activities of α-crystallin preincubated at different temperatures were found to be different and could be correlated with its change in quaternary structure as determined by the fluorescence probe ANS (8-anilo-1-naphthalene sulfonate). α-Crystallin can inhibit the aggregation of γ-crystallin from UV irradiation at room temperature, and the preheated α-crystallins provide more protection than the native one. Upon irradiation by UV light, α-crystallin gradually lost its ability to protect β-crystallin against thermal aggregation. The loss of the chaperone efficacy of α-crystallin to protect other lens proteins may shed light on human cataract formation induced by long-term exposure to UV irradiation.

KEY WORDS: α-Crystallin; procine crystallins; chaperone activity; 8-anilo-1-naphthalene sulfonate (ANS); thermal aggregation; UV irradiation.

1. INTRODUCTION

α-Crystallin, a major protein of the lens, has been shown to prevent thermal aggregation of crystallins and other proteins like a molecular chaperonin (Horwitz, 1992, 1993). Whether this activity has any functional significance for the lens is not yet clear. The chaperone activity of α-crystallin was also shown to decrease on treatment with calpain II *in vitro* and in selenite-induced cataract *in vivo* (Kelly *et al.*, 1993). The transparency of the eye lens may thus depend on the protective role of α-crystallin, i.e., its chaperone activity, and the status of chaperonin function in α-crystallin may be relevant to the loss of transparency and crystallin aggregation associated with cataract.

Since the eye lens is constantly exposed to visible and UV light irradiation, lens proteins supposedly encounter more photochemical insult than thermal stress *in vivo*. It was shown that β- and γ-crystallins, the other two major lens proteins, can undergo photochemical cross-linking in neutral buffer solutions at room temperature when exposed to UV irradiation, subsequently resulting in crystallin aggregation. On the other hand, α-crystallin solution remained clear without turbidity upon exposure to UV irradiation under the same conditions (Hott and Borkman, 1993). Thus the chaperonin role of α-crystallin in the photochemical damage of water-soluble lens proteins may be similar to that in thermal denaturation and aggregation of some other proteins and enzymes (Horwitz, 1993). However, it is of interest to note that α-crystallin provided less protection in photochemical experiments on γ-crystallin at ambient temperature than that in

[1] Department of Ophthalmology, Chang-Gung Memorial Hospital, Taipei, Taiwan.
[2] Laboratory of Crystallin Research, Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.
[3] Institute of Biological Chemistry, P.O. Box 23-106, Academia, Taipei, Taiwan.
[4] To whom correspondence should be addressed; e-mail: shchiou@bc.ntu.edu.TW.

0277-8033/97/0500-0283$12.50/0

thermal aggregation experiments (Raman and Rao, 1994; Borkman *et al.*, 1996). In this study we investigate the photoaggregation of γ-crystallin under UV irradiation in the presence of various preheated α-crystallin solutions in order to gain some insight into the difference in molecular mechanism of chaperone activity of α-crystallin under thermal and photochemical conditions.

2. MATERIALS AND METHODS

2.1. Preparation of Crystallins from Procine Lenses

α-, β-, and γ-Crystallins were isolated from extracts of procine lenses according to the procedure described in Chiou and Azari (1989). α-Crystallins were also isolated from one normal human lens (20-year-old) and one senile cataract lens (65-year-old), provided by ophthalmologic surgery (J.-S.L.).

2.2. Modification of α-Crystallin by Preheating or by UV Irradiation

α-Crystallins (0.3 mg/ml) used in the photochemical experiments were preincubated at different temperatures (50 and 60°C) for 20 min, followed by equilibration at room temperature for 12 hr prior to the irradiation experiments. In thermal aggregation experiments, α-crystallin solutions (0.1–1 mg/ml) were exposed to 280-nm UV light by placing samples in a quartz curvette of a Hitachi F4010 fluorescence spectrophotometer (using 20-nm bandpass) for various periods (5–60 min). The fluorescence intensities of the UV-irradiated α-crystallin samples were then monitored by excitation set at 290 nm. The chaperone activity against thermal aggregation of β-crystallin at 60°C by UV-irradiated α-crystallin samples (0.1 mg/ml) was also studied.

2.3. Fluorescence of 8-Anilo-1-Naphthalene Sulfonate (ANS) Bound to α-Crystallins

ANS, a hydrophobic fluorescence probe used for estimation of the surface hydrophobicity of protein solutions, was freshly prepared (0.1 mM) and added to the α-crystallin solutions preincubated at 50 and 60°C and equilibrated at room temperature for 2 hr before fluorescence measurements. Fluorescence spectra of α-crystallin solutions were recorded with a Hitachi F4010 fluorescence spectrophotometer by setting the excitation wavelength at 365 nm with a light slit set at 5 nm for both excitation and emission modes.

2.4. Assays for Crystallin Aggregation Induced by UV Irradiation and Thermal Heating

Photoaggregation of γ-crystallin (1 mg/ml) or thermal aggregation of β-crystallin (0.37 mg/ml) were monitored in the elution buffer of a TSK gel-filteration column used for crystallin isolation (Chiou and Azari, 1989) in the absence or presence of α-crystallin. The sample solutions were exposed under UV lamp (254 nm) or incubated at 60°C. The extent of protein aggregation or turbidity was measured as a function of time by light scattering of solutions as estimated by absorbance at 360 nm with a Jasco 7800 UV/VIS spectrophotometer.

2.5. Protein Concentration Determinations

The concentration of γ-crystallin used in the experiments was 1 mg/ml, whereas that of β-crystallin was 0.30–0.37 mg/ml and α-crystallin 0.1–0.3 mg/ml. All concentrations of crystallin solutions were estimated from their respective extinction coefficients at 280 nm (0.83, 2.23, and 2.26 for α-, β-, and γ-crystallins, respectively, at 1 mg/ml) (Chiou *et al.*, 1981).

3. RESULTS AND DISCUSSION

It is well known that some proteins can act as molecular chaperonins to mediate the correct assembly of other polypeptides (Hendrick and Hartl, 1993). However, the detailed mechanism for the protective role of "chaperoning" function has not been completely elucidated. A previous study suggested that hydrophobic interactions may play a major role in the chaperone action of α-crystallin (Das and Surewicz, 1995), a recently discovered chaperonin present abundantly in the animal lenses (Horwitz, 1993). α-Crystallin was shown to undergo an irreversible conformational transition with a marked increase in surface hydrophobicity upon

heating (Das and Surewicz, 1995). In this report, we investigate the photoaggregation of γ-crystallin in the presence of preheated α-crystallins and monitor the structural change of α-crystallins induced by heating using a hydrophobic fluorescent probe in order to provide some insights into molecular mechanisms of chaperone activity of α-crystallin against UV-induced photodamage as compared to thermal aggregation of other lens proteins.

3.1. Structural Change of α-Crystallin upon Heating as Probed by Fluorescent ANS

The fluorescence quantum yield of ANS depends strongly on the polarity of the environment, increasing dramatically upon binding to hydrophobic sites of proteins (Kato and Nakai, 1980). In Fig. 1 the fluorescence intensity of free ANS in an aqueous buffer is very weak, with a maximum locating at 525 nm. In the presence of α-crystallin, the maximum of the emission spectrum shifts to 463 nm and there is a strong enhancement of the fluorescence quantum yield for ANS. It is also noteworthy that the fluorescent intensity of ANS increases significantly upon binding of ANS with α-crystallin at elevated preincubation temperatures, indicative of a gradual exposure of the hydrophobic surface of α-crystallin upon heating.

3.2. Chaperone Activity of α-Crystallin to Prevent Photoaggregation of γ-Crystallin

The above observation for the fluorescence intensity change in ANS-bound α-crystallin is in general agreement with the previous report indicating that perturbation of the quaternary structure of α-crystallin by denaturing agents of nonthermal mode such as 3 M urea can result in enhanced chaperone activity as compared with the native crystallin, which does not prevent photoaggregation at lower ambient temperatures (Raman and Rao, 1994).

Therefore α-crystallin indeed undergoes a subtle and yet distinct conformational transition as revealed by a significant change of fluorescent emission spectra of ANS-bound crystallin by preincubation at higher temperatures, which also correlates with its increased chaperone activity against the photoaggregation of γ-crystallin by UV irradiation at 254 nm (Fig. 2). In this study, γ-crystallin aggregates upon exposure to UV light in the absence of α-crystallin. Although α-crystallin

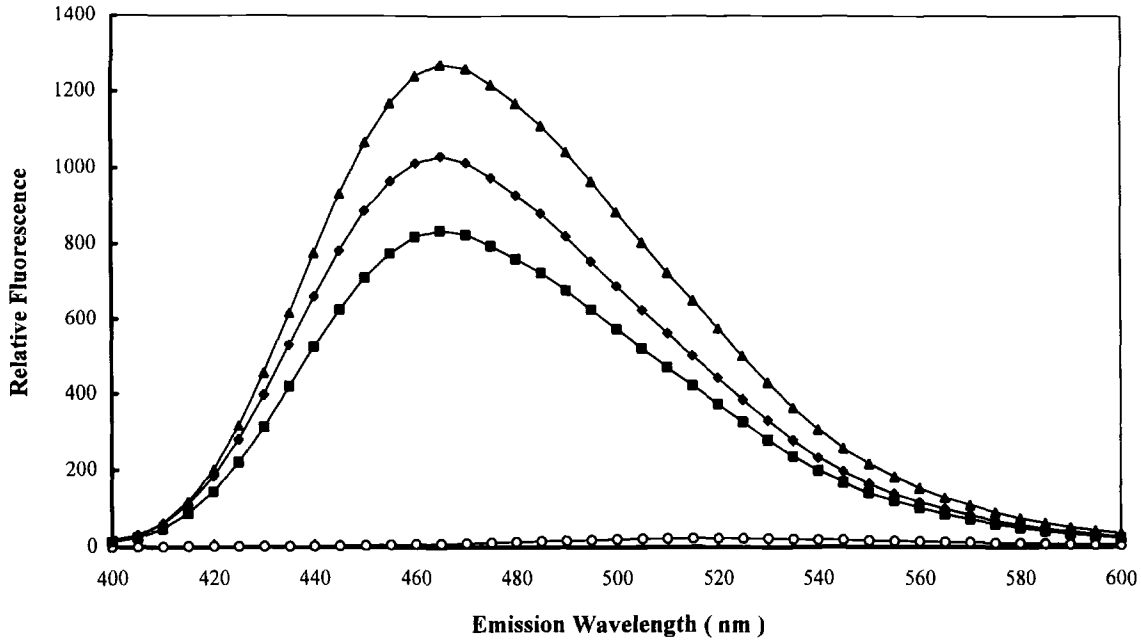

Fig. 1. Comparison of fluorescence emission spectra of ANS in the presence of α-crystallin preincubated at different temperatures. The fluorescence spectra were recorded with a Hitachi F4010 fluorescence spectrophotometer by setting the excitation wavelength at 365 nm with a light slit set at 5 nm for both excitation and emission modes. ANS in control buffer solution (-○-); ANS with native α-crystallin without heating (-■-); ANS with α-crystallin preheated at 50°C (-◆-); and ANS with α-crystallin preheated at 60°C (-▲-).

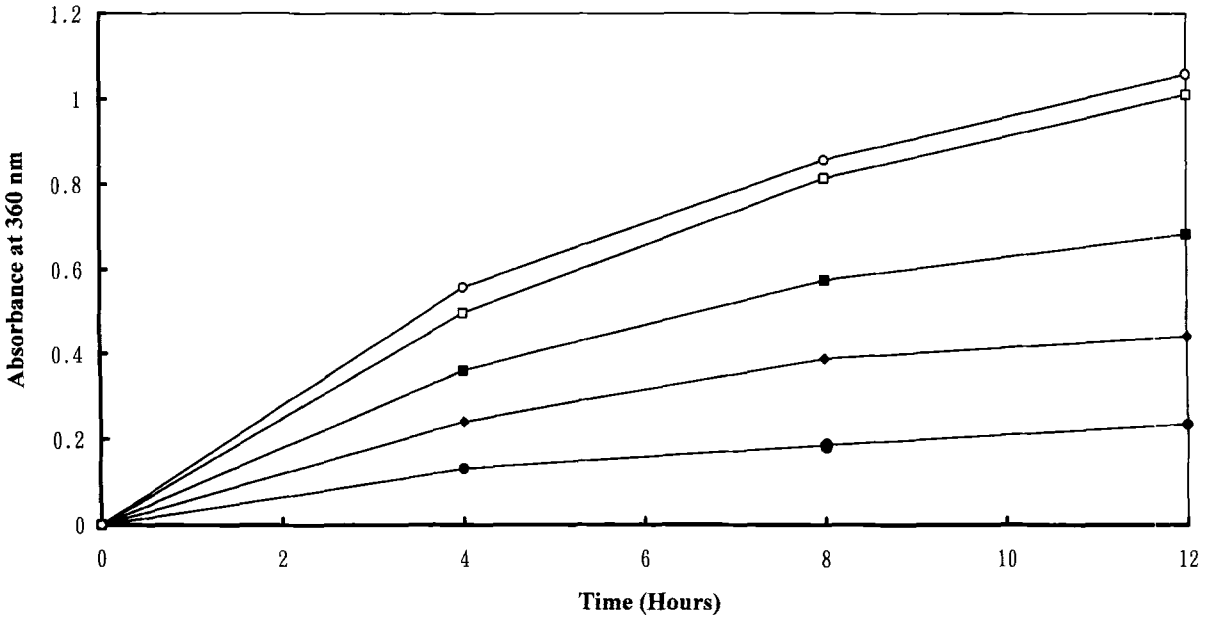

Fig. 2. Photoaggregation of γ-crystallin irradiated with a 254-nm UV lamp in the presence of α-crystallin preincubated at different temperatures. The concentration of γ-crystallin used in these experiments was 1 mg/ml, whereas that of α-crystallin was 0.1 and 0.3 mg/ml. All concentrations of crystallin solutions used in the experiments were estimated from their respective extinction coefficients at 280 nm (0.83, 2.23, and 2.26 for α-, β-, and γ-crystallins, respectively, at 1 mg/ml). γ-Crystallin with α-crystallin (0.3 mg/ml) preheated at 60°C (-●-); γ-crystallin with α-crystallin (0.3 mg/ml) preheated at 50°C (-◆-); γ-crystallin with native α-crystallin (0.3 mg/ml) without heating (-■-); γ-crystallin only (-□-); and γ-crystallin with native α-crystallin (0.1 mg/ml) (-○-).

was previusly shown to prevent heat-induced aggregation of γ-crystallin (Horwitz, 1992), it does not prevent the photoaggregation in our experiment at a low concentration of 0.1 mg/ml and very poorly at 0.3 mg/ml (Fig. 2). Similar to the result of a previous report (Walker and Borkman, 1989), α-crystallin can inhibit the photoaggregation of γ-crystallin at elevated temperatures and the preheated α-crystallins in general provide more protection than the native one incubated at room temperature. The results point strongly to the fact that the conformational change as reflected in the exposure of hydrophobic domain(s) inside α-crystallin may be a prerequisite for chaperone activity observed at higher temperatures or in the presence of denaturants.

3.3. Chaperone Activity of α-Crystallin to Prevent Thermal Aggregation of β-Crystallin

With regard to the difference between crystallin aggregation induced by heating or UV irradiation, we have followed the Trp fluorescence spectra of native α-crystallin by irradiating α-crystallin with UV light at 290 nm for different periods of 5–60 min. As indicated in Fig. 3, upon exposure of α-crystallin solutions to UV light, a significant decrease of Trp fluorescence in α-crystallin is observed, reflective of some destruction of Trp residues or change of Trp microenvironment by UV irradiation. In contrast, α-crystallin preheating at 60°C for 1 h did not lose its Trp fluorescence when compared with that of native protein without heating. It is known that the fluoroescence of α-crystallin when excited at 290 nm is dominated by the fluorescent emission (emission maximum at about 340 nm) from Trp-9 (αA and αB subunits) and from Trp-60 (αB only) (Augusteyn *et al.*, 1993). Therefore the decrease in Trp fluorescence upon UV irradiation observed here is probably due to some photochemical destruction or exposure of Trp residues, which were presumably affected much less by thermal heating.

As shown in Fig. 4, the rapid aggregation of β-crystallin (0.37 mg/ml) during incubation at 60°C is completely inhibited in the presence of native

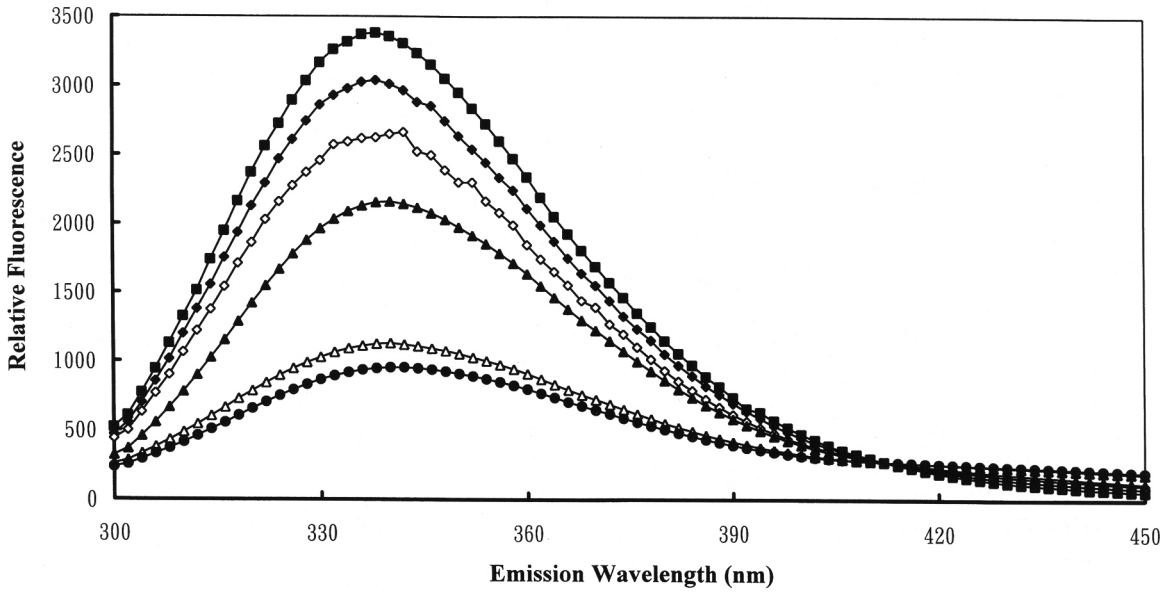

Fig. 3. Change of tryptophan fluorescence spectra of α-crystallin upon exposure to UV irradiation at 280 nm. The fluorescence spectra were recorded by setting the excitation wavelength at 290 nm. The concentration of α-crystallin used in the experiments was 1 mg/ml. The emission spectra from top to bottom correspond to those crystallin solutions irradiated at UV 280 nm for 0, 5, 10, 15, 30, and 60 min, respectively, with the solution of the longest exposure showing the lowest fluorescence.

α-crystallin (0.1 mg/ml). A correlation is also revealed between the reduction in Trp fluorescence of α-crystallin by UV irradiation (Fig. 3) and the loss of its ability to prevent the thermal aggregation of β-crystallin (Fig. 4).

There is still much debate on controversial

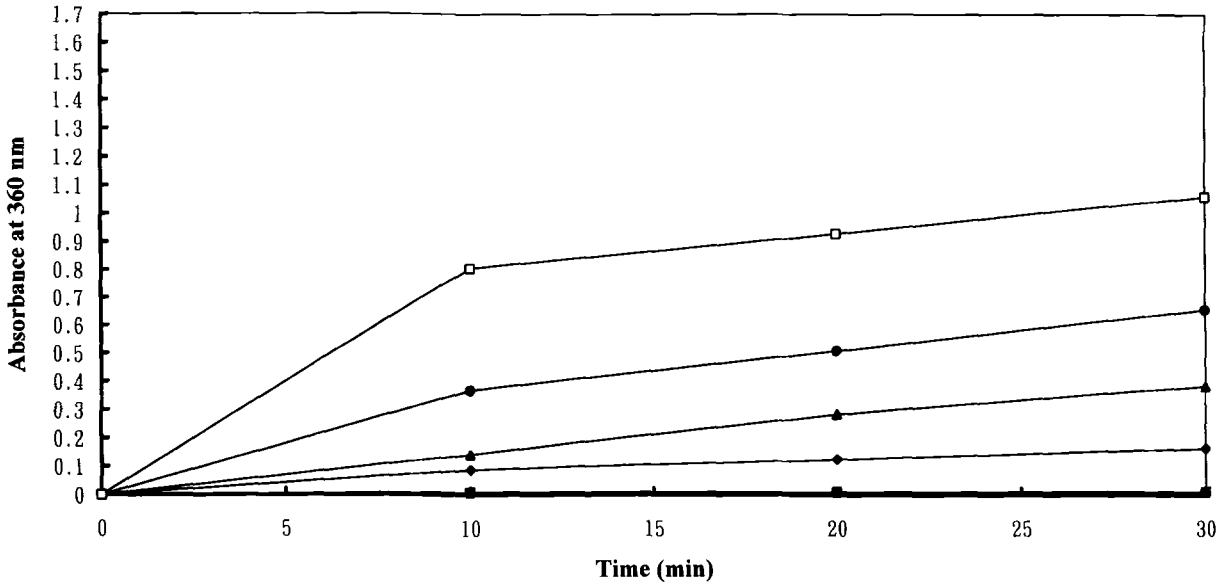

Fig. 4. Thermal aggregation of β-crystallin at 60°C in the presence of α-crystallin irradiated with UV at 280 nm. The concentration of β-crystallin used in these experiments was 0.37 mg/ml, whereas that of α-crystallin was 0.1 mg/ml. All concentrations of crystallin solutions used in the experiments were estimated from their respective extinction coefficients at 280 nm (0.83, 2.23, and 2.26 for α-, β-, and γ-crystallins, respectively, at 1 mg/ml). β-Crystallin with native α-crystallin (-■-); β-crystallin with α-crystallin irradiated for 5 min (-◆-); β-crystallin with α-crystallin irradiated for 15 min (-▲-); β-crystallin with α-crystallin irradiated for 60 min (-●-); and β-crystallin only (-□-).

results regarding the chaperone activity of α-crystallin to prevent UV-induced protein aggregation below 30°C (Raman and Rao, 1994; Borkman *et al.*, 1996). The discrepancy seemed to lie in the concentrations of α-crystallin used in the photochemical experiments employing UV irradiation (Fig. 2). In general a higher concentration of α-crystallin is needed for the protection of lens proteins against UV light than against thermal heating.

In thermal denaturation of α-/γ-crystallin mixtures, Horwitz (1992, 1993) found the stoichiometry of α/γ ratio of about 1:10–20 (mol/mol). However, α-crystallin provided little photochemical protection for γ-crystallin at room temperature based on this ratio (Raman and Rao, 1994; and our published data). The recent report of Borkman *et al.* (1996) corroborated this observation by showing that the molar ratio of α-/γ-crystallin mixture needed for effective protection of γ-crystallin solution from photochemical opacification was higher than that from thermal studies. In order to potentiate the chaperone activity of α-crystallin *in vivo*, it is conceivable that α-crystallin must somehow unfold partially its quaternary structure either by heat or by some other structure-disturbing agents as revealed in the *in vitro* studies. In the present study the increase in the surface hydrophobicity of α-crystallin induced by elevated temperature of nonphysiological nature (>50°C) can indeed increase the chaperone activity of α-crystallin. It remains to be investigated whether the same enhancement of chaperone function can be modulated or achieved by some other innocuous agents under more physiological conditions.

4. CONCLUSION

Age-related changes in human eye lenses such as formation of high-molecular-weight protein aggregates and increased amounts of degraded polypeptides have often been found to be accompanied by a gradual loss of enzymatic activities and other biological functions in clear lenses (Srivastava, 1988). In particular, α-crystallins during aging were shown to form intermolecular cross-linking triggered by photooxidation of some aromatic amino acid residues of lenses crystallins (Goosey *et al.*, 1980). Since the role of photoinduced damages in cataract is well documented (Garcia-Castineiras *et al.*, 1978), it is reasonable to assume that light-induced crystallin changes may be more relevant to human cataract formation caused by age-dependent modifications in major classes of lens proteins, especially α-crystallin with genuine chaperone activity reported recently (Horwitz, 1992). We have found some age-dependent change in the chaperone activity of α-crystallins obtained from a young and normal lens as compared to an old and cataractous lens (Fig. 5). It is evident that α-crystallin of a cataractous lens possesses a lower chaperone activity than that of a normal lens and the activity persists even at an old and diseased state.

α-Crystallin is a multimeric protein composed of two polypeptides, αA and αB, that have considerable sequence homology and are held together noncovalently to form aggregates ranging from 700 to 1000 kDa, of which the quaternary structure has not been determined; different models have been proposed to account for the "chaperoning" property of this unique class of lens proteins (Groenen *et al.*, 1994). It was previously demonstrated that a remarkable correlation existed between the reduction in α-crystallin fluorescence during UV irradiation and the loss of its ability to protect phosphoglycerate kinase against thermal aggregation (Schauerte and Gafni, 1995). In this study we have also found that a similar correlation can be applied to the chaperonin function of α-crystallin in protecting other lens proteins (Figs. 3 and 4). Since a loss of tryptophan fluorescence in intact eye lenses has been demonstrated to occur upon exposure to UV light for *in vivo* experiments as well as during the aging process (Fujimori, 1982), it is conceivable that the enhanced rate of cataract formation with aging is related to the gradual loss of the chaperone activity of α-crystallin in old lenses as demonstrated here.

In this work, we have studied the effects of preheating and UV irradiation on the chaperone activity of α-crystallin to protect β- or γ-crystallins against heat- or UV-induced crystallin aggregation. α-Crystallin can enhance its chaperone activity against photodamage by UV irradiation after partially unfolding its quaternary structure by preincubation at higher temperatures. It is also shown that α-crystallin can gradually lose its chaperone activity with aging, probably through an accumulative event of long-term exposure to UV light.

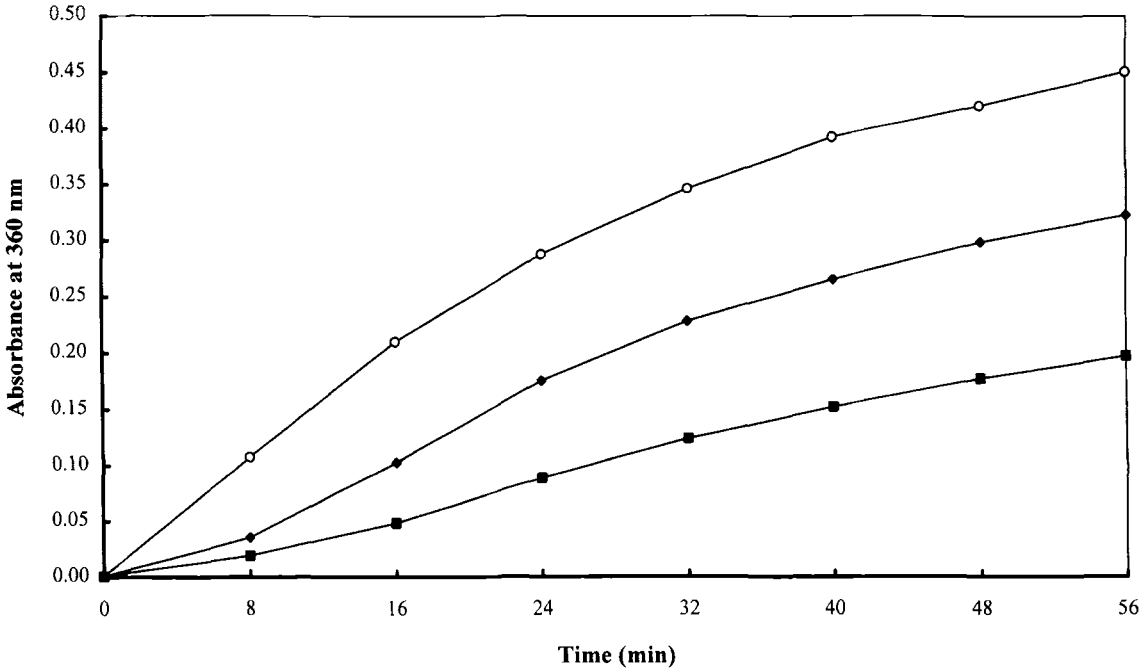

Fig. 5. Thermal aggregation of β-crystallin at 60°C in the presence of α-crystallins isolated from normal and cataractous lenses. The concentration of β-crystallin used in these experiments was 0.3 mg/ml, whereas that of α-crystallin was 0.003 mg/ml. β-Crystallin with native α-crystallin from a normal young lens (-■-); β-crystallin with α-crystallin from a cataractous old lens (-◆-); and β-crystallin only (-○-).

ACKNOWLEDGMENTS

This work was supported in part by Academia Sinica and the National Science Council, Taipei, Taiwan. We are grateful for the donation of human lens samples by Chang-Gung Memorial Hospital.

REFERENCES

Augusteyn, R. C., Ghiggino, K. P., and Putilina, T. (1993). *Biochem. Biophys. Acta* **1162,** 61–71.
Borkman, R. F., Knight, G., and Obi, B. (1996). *Exp. Eye Res.* **62,** 141–148.
Chiou, S. H., and Azari, P. (1989). *J. Protein Chem.* **8,** 1–17.
Chiou, S.-H., Chylack, L. T., Jr., Tung, W. H., and Bunn, H. F. (1981). *J. Biol. Chem.* **256,** 5176–5180.
Das, K. P., and Surewicz, W. K. (1995). *FEBS Lett.* **369,** 231–325.
Fujimori, E. (1982). *Exp. Eye Res.* **34,** 381–384.
Garcia-Castineiras, S., Dillan, J., and Spector, A. (1978). *Science* **199,** 897–899.
Goosey, J. D., Zigler, J. S., and Kinoshita, J. H. (1980). *Science* **208,** 1278–1280.
Groenen, P. J. T. A., Merk, K. B., De Jong, W. W., and Bloemendal, H. (1994). *Eur. J. Biochem.* **225,** 1–19.
Hendrick, J. P., and Hartl, F. U. (1993). *Annu. Rev. Biochem.* **62,** 349–384.
Horwitz, J. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 10449–10453.
Horwitz, J. (1993). *Invest Ophthalmol. Vis. Sci.* **34,** 10–22.
Hott, J. L., and Borkman, R. F. (1993). *Photochem. Photobiol.* **57,** 312–317.
Kato, A., and Nakai, S. (1980). *Biochim. Biophys. Acta* **624,** 13–20.
Kelly, M. J., David, L. L., Iwasaki, N., Wright, J., and Sheaven, T. R. (1993). *J. Biol. Chem.* **268,** 18844–18849.
Raman, B., and Rao, C. M. (1994). *J. Biol. Chem.* **269,** 27264–27268.
Schauerte, J. A., and Gafni, A. (1995). *Biochem. Biophys. Res. Commun.* **212,** 900–905.
Srivastava, O. P. (1988). *Exp. Eye Res.* **47,** 525–543.
Walker, M. L., and Borkman, R. F. (1989). *Exp. Eye Res.* **48,** 375–383.