ACADEMIC PRESS

Biochemical and Biophysical Research Communications 297 (2002) 309–316

BBRC

www.academicpress.com

C-terminal lysine truncation increases thermostability and enhances chaperone-like function of porcine αB-crystallin☆

Jiahn-Haur Liao,[a] Jiahn-Shing Lee,[b] and Shyh-Horng Chiou[a,c,*]

[a] *Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan*
[b] *Department of Ophthalmology, Chang-Gung Memorial Hospital, Taipei, Taiwan*
[c] *Laboratory of Crystallin Research, P.O. Box 23-106, Institute of Biological Chemistry, Academia, Nankang, Taipei 11529, Taiwan*

Received 7 August 2002

Abstract

The carboxyl-terminal segment of α-crystallin, a major lens protein of all vertebrates, has a short and flexible peptide extension of about 20 amino acid residues that are very susceptible to proteolytic truncation and modifications under physiological conditions. To investigate its role in crystallin aggregation and chaperone-like activity, we constructed a mutant of porcine αB-crystallin with C-terminal lysine truncated end, which unexpectedly showed better chaperone-like function than wild-type αB-crystallin. From circular dichroism (CD) spectra, we show that the mutant possesses similar secondary and tertiary structures to those of native purified and recombinant αB-crystallins. Analytical ultracentrifugation revealed that the truncated mutant was smaller than wild-type αB-crystallin in aggregation size and mass. The observed higher thermostability and anti-thermal aggregation propensity of the truncated αB-crystallin mutant than wild-type αB-crystallin are in contrast to the prevailing notion that mutations at the C-terminal lysines of αB-crystallin result in substantial loss of chaperone-like activity, despite the overall preservation of secondary structure. The detailed characterization of the C-terminal deletion mutants may provide some deeper insight into the chaperoning mechanism of the structurally related small heat-shock protein family.

Keywords: αB-crystallin; C-terminal truncation mutant; Chaperone activity; Thermostability; Circular dichroism; Small heat-shock proteins

α-Crystallin extracted from mammalian eye lenses exists in solution as polydisperse aggregates ranging from 600 to 1200 kDa with an average molecular mass of approximately 800 kDa [1]. There are two α-crystallin genes that encode two α-crystallin subunits known as αA and αB, resulting in two polypeptides of about 20 kDa in size and 55–60% sequence similarity. αA-crystallin is usually the more abundant subunit in the lens, although the αA/αB ratio varies considerably among species. They were previously regarded as lens-specific proteins and of exclusively structural nature. Ingolia and Craig first reported that heat-shock proteins of *Drosophila* showed sequence similarity to mammalian α-crystallin [2]. Later, it was demonstrated that α-crystallin could function like heat-shock proteins by acting as molecular chaperones in suppressing the aggregation of other proteins in vitro [3]. αA-crystallin is highly specialized for expression in the lens [4]; but αB-crystallin is shown to be a functional small heat-shock protein, which is ubiquitously present in various tissues [5–7].

The small heat-shock proteins (sHSPs) form a structurally divergent protein family with members present in archaea, bacteria, and eukarya [8,9]. All members of the sHSP family are characterized by the presence of a homologous sequence of about 80 residues, which has been called the "α-crystallin domain" [2,10,11]. This domain is preceded by an N-terminal domain, which is highly variable in size and sequence, followed by a short and poorly conserved C-terminal extension. It was found that C-terminal truncated αA-crystallin by trypsin digestion showed a decreased ability to protect proteins from heat-induced aggregation using an in vitro assay

☆ *Abbreviations:* αB-crystallin, homoaggregate formed by association of αB crystallin subunits; sHSPs, small heat-shock proteins; CD, circular dichroism; ANS, 8-anilino-1-naphthalene sulfonate; PBS, phosphate-buffered saline.

* Corresponding author. Fax: +886-2-26530014.
E-mail address: shchiou@gate.sinica.edu.tw (S.-H. Chiou).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)02185-X

[12]. According to NMR analyses, the C-terminal 8 and 10 residues of αA- and αB-crystallin, respectively, appear to adopt a conformation as a solvent-exposed random coil [13]. The presence of an exposed C-terminal extension was in accord with the observation that the last 20 or so residues of the α-crystallin are especially liable to truncations and modifications [14–18]. A mutant of αA-crystallin in which hydrophobicity was introduced into the C-terminal extension was shown to behave significantly less flexible and a corresponding reduction in solubility, heat stability, and chaperone activity when compared with the wild-type protein [19]. A mutant of mouse heat-shock protein (Hsp25) without this extension was also constructed and shown to exhibit a chaperone activity comparable to that of wild-type Hsp25 in thermal aggregation assay using citrate synthase as substrate, but does not stabilize α-lactalbumin against precipitation following reduction with dithiothreitol [20]. Overall, previous structural studies suggested that a highly flexible C-terminal extension in mammalian sHSPs or the related αA or αB crystallins appeared to be a prerequisite for full chaperone activity.

We have previously cloned and expressed porcine αB-crystallin [21], useful for site-specific mutagenesis to derive the structure–function correlation of this molecular chaperone. In this report, we have prepared C-terminal lysine truncated mutants of αB-crystallin, examined and compared the structural and functional properties of these lysine-deleted mutants with wild-type recombinant αB, and purified αB crystallin from native lens by various biophysical methods. The results obtained from these combined biophysical approaches provide some insight into the structural basis of C-terminal lysine residues in relation to the chaperone activity of intact αB-crystallin.

Materials and methods

Isolation of lens crystallins. Porcine lenses were decapsulated and homogenized in a buffer containing 50 mM Tris–HCl, 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol, and 0.02% sodium azide, pH 8.0. After centrifugation at 27,000*g* for 30 min, the supernatant was applied to a column packed with TSK HW-55(F) gel and eluted at 25 ml/h. Five well-resolved peaks were obtained and identified as HMα-, α-, βH-, βL-, and γ-crystallins based on SDS–PAGE.

Construction of expression vector. The cDNA coding for porcine αB-crystallin was cloned and expressed as described previously [21]. The mutant cDNA fragments were made by polymerase chain reaction.

The same forward primer (5′-GCCATATGGACATCGCCATC CACCACC-3′) was used for recombinant wild-type αB and lysine-deleted mutant (T1, minus two lysine residues) crystallins.

The reverse primers used for wild-type αB and T1 were: 5′-GCAAGCTTCTACTTCTTGGGGGCTGCAG-3′ (WT-αB) and 5′-GCAAGCTTCTAGGGGGCTGCAGTGACAGC-3′ (T1), respectively. The PCR products were double-digested with *Nde*I and *Hin*dIII and then ligated into *Escherichia coli* expression vector pET21a(+).

Expression and purification of porcine αB-crystallin and its mutants. *Escherichia coli* strain BL21(DE3) was transformed using above-mentioned expression constructs. The cells were incubated at 37 °C until the culture reached an optical density of 0.6 at 600 nm. Enhanced protein expression was assisted by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Four hours after incubation, cells were harvested, resuspended in lysis buffer (20 mM Tris–HCl, 25 mM NaCl, 1 mM EDTA, and 8 M urea, pH 8), and lysed by ultrasonication. After ultrasonication, the mixture was then centrifuged for 20 min at 20,000*g* to remove any remaining insoluble debris. Soluble recombinant proteins were purified by a TSK HW-55 gel filtration column and followed by reverse-phase HPLC (C4). The purified recombinant αB-crystallin (WT-αB) and T1 mutant were then lyophilized.

Refolding and reconstitution of αB-crystallin and its mutants. Native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins were solubilized in 8 M urea individually and then loaded onto a gel filtration column (TSK HW-55 gel). The separated protein fractions were then collected and concentrated. The protein concentrations were determined by absorbance measurements using extinction coefficients that are calculated from amino acid sequence data of each protein [22].

Circular dichroism spectra. Circular dichroism spectra were performed on a JASCO J-715 spectropolarimeter. Protein concentrations were 1.8×10^{-5} M (far-UV region) and 3.6×10^{-5} M (near-UV region) in the buffer of 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 3 mM KCl and saturated with NaF, pH 7.4. The far-UV CD spectra were the mean of five accumulations with a 0.1 cm light path. The near-UV CD spectra were the mean of 10 accumulations with a 1 cm light path.

Fluorescence spectroscopy. The intrinsic fluorescence spectra were recorded with a Hitachi F4010 fluorescence spectrophotometer by setting the excitation wavelength at 295 nm, a light slit of 5 nm for both excitation and emission modes. The concentrations of native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins was adjusted to 6.5 μM in the buffer of 10 mM $NaHPO_4$, 2 mM KH_2PO_4, 3 mM KCl, and 0.1 M NaCl, pH 7.4. Surface hydrophobicity of porcine native αB (N-αB), WT-αB, and T1 mutant crystallins were also analyzed using the fluorescent probe 8-anilino-1-naphthalene sulfonate (ANS). For each sample, 10 μl ANS in methanolic stock solution (0.1 M) was added to 1 ml of 0.3 mg/ml protein solution and incubated at indicated temperatures for 1 h, respectively. The extrinsic fluorescence spectra were then measured with a Hitachi F4010 fluorescence spectrophotometer by setting the excitation wavelength at 395 nm, a light slit of 5 nm for both excitation and emission modes.

Analytical ultracentrifugation analysis. The molecular weight of these crystallins under various conditions was determined by a Beckman XL-A analytical ultracentrifuge (Beckman Instruments, Fullerton, CA) with an An60Ti rotor. Sedimentation velocity was performed at 25,000 rpm with standard double-sector aluminum centerpieces at 20 °C. The UV absorption of the cells was scanned every 5 min for 2 h. The data were then analyzed with the software provided by the manufacturer and the software SEDFIT [23]. Sedimentation equilibrium was performed at 20 °C in a six-channel epon centerpiece with the centrifugation set at 4800 rpm for 18 h. The data were also analyzed with a software provided by Beckman. All samples were visually checked for clarity after ultracentrifugation and no precipitation was observed.

Assays of in vitro chaperone-like activity. The chaperone-like activities of porcine native αB (N-αB), recombinant WT-αB, and T1 mutant αB-crystallins were studied by two assays. Porcine βL-crystallin was used as substrate in the assay at 58 °C. The final concentration of βL-crystallin was 6.8 μM in the buffer of PBS. The molar ratio was 1:1 (βL-crystallin: chaperone crystallin). In γ-crystallin aggregation assay at 70 °C, porcine γ-crystallin was used as substrate in final concentrations of 5 and 2.5 μM using PBS buffer. The molar ratio was 1:1 and 1:2 (γ-crystallin:chaperone crystallin) when 5 or 2.5 μM porcine γ-crystallin was used, respectively.

Thermal stability of porcine αB-crystallin and its mutants. The assayed proteins were in a PBS buffer containing 10 mM Na_2HPO_4,

2 mM KH_2PO_4, 3 mM KCl, and 0.1 M NaCl, pH 7.4. Each protein sample with an identical concentration of 36 μM was heated in a temperature range of 20–80 °C, continuously measuring turbidity at OD 360 nm. The anti-thermal aggregation capabilities of porcine native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins were assayed at 70 °C. All three crystallins in a concentration of 7.2 μM were dissolved in a buffer containing 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 3 mM KCl, and 0.1 M NaCl, pH 7.4. For each protein, triplicate samples were incubated at 70 °C for 10 min and the turbidities as measured by light scattering at OD 360 nm were measured before and after incubation.

Results and discussion

Recent literature abounds with reports characterizing a variety of α-crystallin mutants and very often with inconsistent and contradictory findings. Since we have previously cloned and expressed porcine αB-crystallin [21], it is very useful to apply site-specific mutagenesis for deriving the structure–function correlation of this molecular chaperone. In this report, we have focused on the role of two C-terminal lysine residues present in αB-crystallin in relation to the chaperone activity of this certified member of sHSPs family. The results obtained on the C-terminal lysine truncated mutants by using various biophysical approaches may provide some insight into the structural basis of C-terminal lysine residues on the flexibility of C-terminal segment and their effect on the chaperone activity of intact αB-crystallin in general.

Expression, purification, and characterization of molecular masses

Escherichia coli strain BL21(DE3) was transformed with expression constructs of αB crystallins. After incubation, cells were harvested and resuspended in lysis buffer and lysed by ultrasonication. Soluble recombinant proteins were purified by TSK HW-55 gel filtration and followed by reverse-phase HPLC (C4). The purified recombinant αB-crystallin and T1 mutant were then lyophilized. The molecular masses of porcine native αB (N-αB), recombinant WT-αB, and T1 mutant (minus two lysine residues) crystallins were analyzed by electrospray mass spectrometry and confirmed to possess correct molecular masses of these subunits (data not shown). Lyophilized native αB (N-αB), recombinant WT-αB, and T1 mutant were dissolved in 8 M urea, refolding in a TSK HW-55(F) gel filtration column. These αB-crystallins and T1 mutant were thus refolded after removal of urea. The purity of these proteins was shown to be >95% by SDS–PAGE.

Circular dichroism spectropolarimetry

The far-UV CD spectra of native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins were very similar. Based on the CD spectra analyzed by the popular algorithm program [24], the secondary structure estimation for these proteins showed mainly β-sheet structure, which was consistent with the prevailing evidence that α-crystallin consists mostly of β-sheet secondary structure [25]. The near-UV CD spectra of native αB (N-αB), recombinant WT-αB, and T1 mutant were also found to be similar (data not shown), indicating that the microenvironments of bulky tryptophan and tyrosine residues in these three crystallins are probably kept in similar milieu.

Intrinsic tryptophan fluorescence spectra and surface hydrophobicity

Intrinsic fluorescence provides information about the local conformation of tryptophan residues inside proteins. Tryptophan residues were excited selectively by irradiation at 295 nm and fluorescence emission spectra were then collected from 300 to 400 nm. The emission maxima were located at 339–340 nm. All three crystallins show similar intrinsic fluorescence spectra (Fig. 1A), indicative of the similar microenvironments of tryptophan residues in three crystallins.

The fluors can be introduced into the molecule to be studied either by chemical coupling or by simple binding. Such extrinsic protein fluorescence generated by the use of such added molecules is sensitive for structural comparison of homologous proteins. The extrinsic flour used in our study is 8-anilino-1-naphthalene sulfonate (ANS). It becomes fluorescent when bound to the hydrophobic area on the surface of various macromolecules. To investigate if there exists any surface hydrophobicity difference among native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins, we probed the surface hydrophobicity of these three crystallins using ANS. The ANS fluorescence spectra (Fig. 1B) of three proteins appear to be similar, which indicate that native purified αB, wild-type αB, and T1 possess similar surface hydrophobicity.

Analytical ultracentrifugation analysis

Further investigation on the quaternary structures of native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins was performed by analytical ultracentrifuge. Both sedimentation velocity and sedimentation equilibrium have been used to characterize the particle sizes of these three crystallins. Sedimentation velocity was performed at 25,000 rpm with standard double-sector aluminum centerpieces at 20 °C. Sedimentation coefficients (S or Svedberg values) were determined with the second moment method of analysis software. The results of 15.8, 14.8, and 13.4 S for native αB (N-αB), recombinant WT-αB, and T1, respectively, were obtained (Fig. 2). Continuous size distribution analysis of

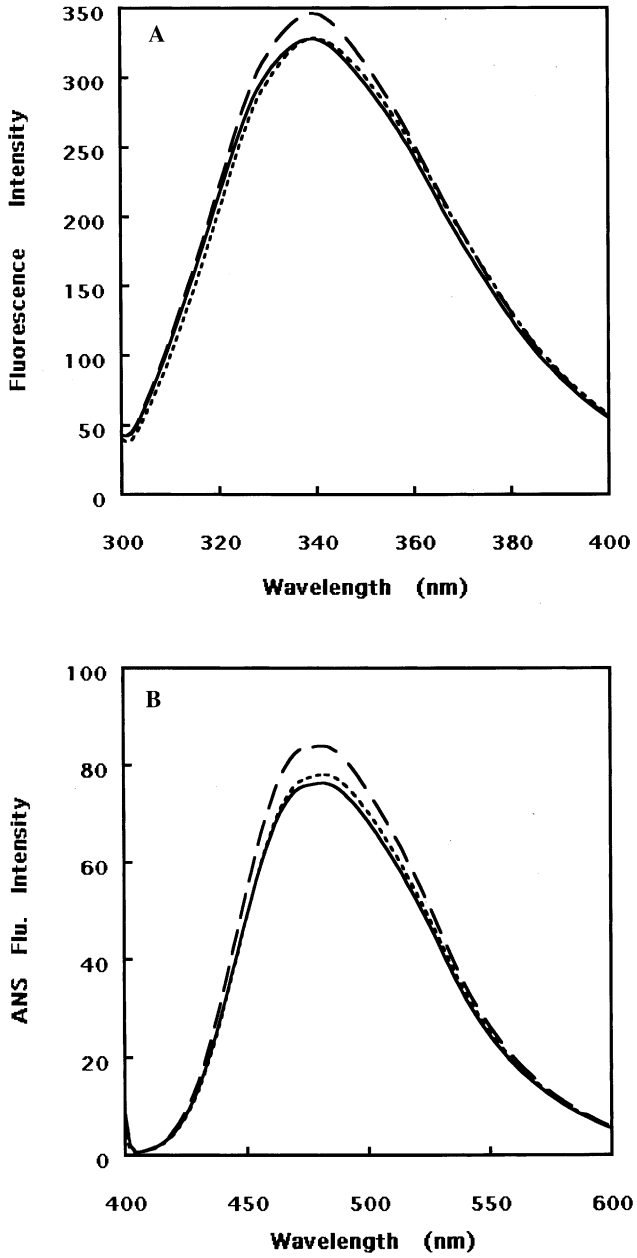

Fig. 1. Fluorescence spectral analysis of native αB, recombinant WT-αB, and T1 mutant crystallins. (A) Intrinsic fluorescence. Spectra were recorded for native αB crystallin (solid line), wild-type αB-crystallin (dash line), and T1 truncated mutant (dot line). Tryptophan residues were excited selectively by irradiation at 295 nm. Fluorescence emission spectra were recorded between 300 and 400 nm. All three proteins show similar intrinsic fluorescence spectra with the same wavelength of emission maxima. (B) Comparison of ANS fluorescence emission spectra for native purified αB-crystallin (solid line), recombinant wild-type αB-crystallin (dash line), and T1 truncated mutant (dot line). The ANS fluorescence spectra of T1 truncated mutant and native αB are similar and slightly lower than those of recombinant wild-type αB-crystallin, which indicate that three crystallins possess similar surface hydrophobicity.

the sedimentation data was fitted by the software SEDFIT program [23]. The fitted sedimentation coefficient distribution curve reveals a broad distribution of

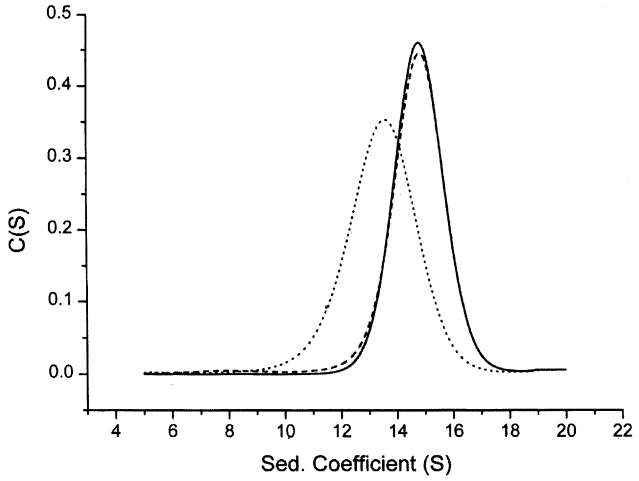

Fig. 2. Sedimentation velocity experiment of native αB, recombinant WT-αB, and T1 mutant. Continuous size distributions of native αB-crystallin (solid line), wild-type αB-crystallin (dash line), and T1 truncated mutant (dot line) are represented as curves of sedimentation coefficient distribution. The sedimentation coefficient distribution of native αB and wild-type αB was between 12 and 18 S, while the distribution of T1 truncated mutant was between 10 and 16 S. All results from different analysis methods show that the sedimentation coefficient of T1 is smaller than those of native αB and recombinant WT-αB crystallins.

crystallin species. The sedimentation coefficient distribution of native αB (N-αB) and recombinant WT-αB crystallins was between 12 and 18 S, while the distribution of T1 mutant was between 10 and 16 S. All results from different analysis methods pointed to the fact that the sedimentation coefficient obtained for T1 is smaller than those of native αB and recombinant WT-αB crystallins. Sedimentation equilibrium at a centrifugation speed of 4800 rpm was performed at 20 °C for 18 h. The molecular weights of native αB, recombinant WT-αB, and T1 are 490,000, 463,000, and 339,000, respectively, in accord with results of sedimentation velocity.

Comparison of the chaperone activity under thermal stress

The differences in the chaperone activity of native αB (N-αB), recombinant WT-αB, and T1 mutant crystallins were characterized in an in vitro aggregation assay using β-crystallin and γ-crystallin as target proteins at 58 °C and 70 °C, respectively. All three proteins show similar chaperone-like activity when porcine βL-crystallin was used as substrate at 58 °C for chaperone activity assay in a molar ratio (chaperone/substrate) of 1:1 (data not shown). However, it was found that although both native and recombinant αB-crystallins lost the protective activity, the C-terminal truncated T1 mutant crystallin retained the protective activity when γ-crystallin was used as substrate at 70 °C in a molar ratio of 1:1 (Fig. 3A). Moreover,

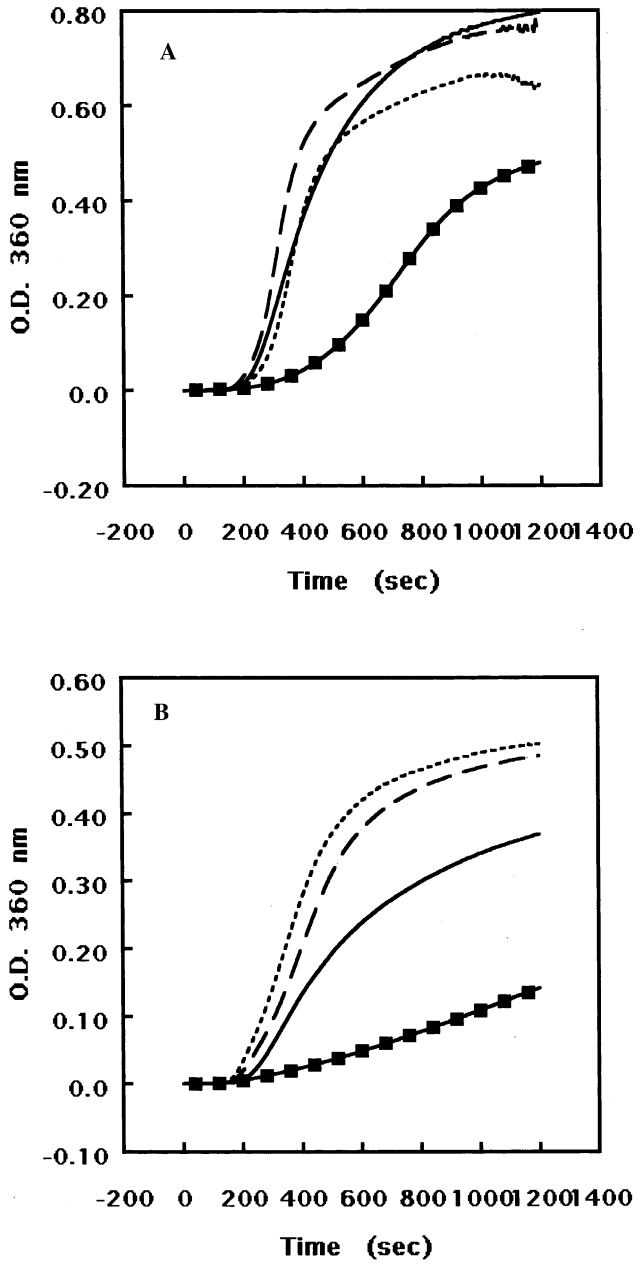

Fig. 3. Comparison of chaperone activities for native αB, recombinant WT-αB, and T1 mutant against thermal aggregation of porcine γ-crystallin at 70 °C. Porcine γ-crystallin was used as substrate in a molar ratio (chaperone/γ-crystallin) of 1:1 (A) and 2:1 (B). The incubation mixture contained γ-crystallin, in the absence (solid line) and presence of native αB-crystallin (dash line), recombinant wild-type αB-crystallin (dot line), and T1 (line with square). Both assays show that T1 mutant protein has the protective activity for γ-crystallin at 70 °C. Note that native αB and recombinant WT-αB are very unstable at 70 °C and turn turbid more quickly than control γ-crystallin as shown in (B).

when we increased the molar ratio for the chaperoning crystallin by decreasing the amount of γ-crystallin, neither native nor recombinant αB-crystallin regained their protective activity. In contrast, T1 truncation mutant showed significantly higher protective activity.

Thermal stability of native purified, wild-type αB-crystallin, and T1 mutant

We study the protein stability by incubating native purified, recombinant wild-type αB-crystallin, and T1

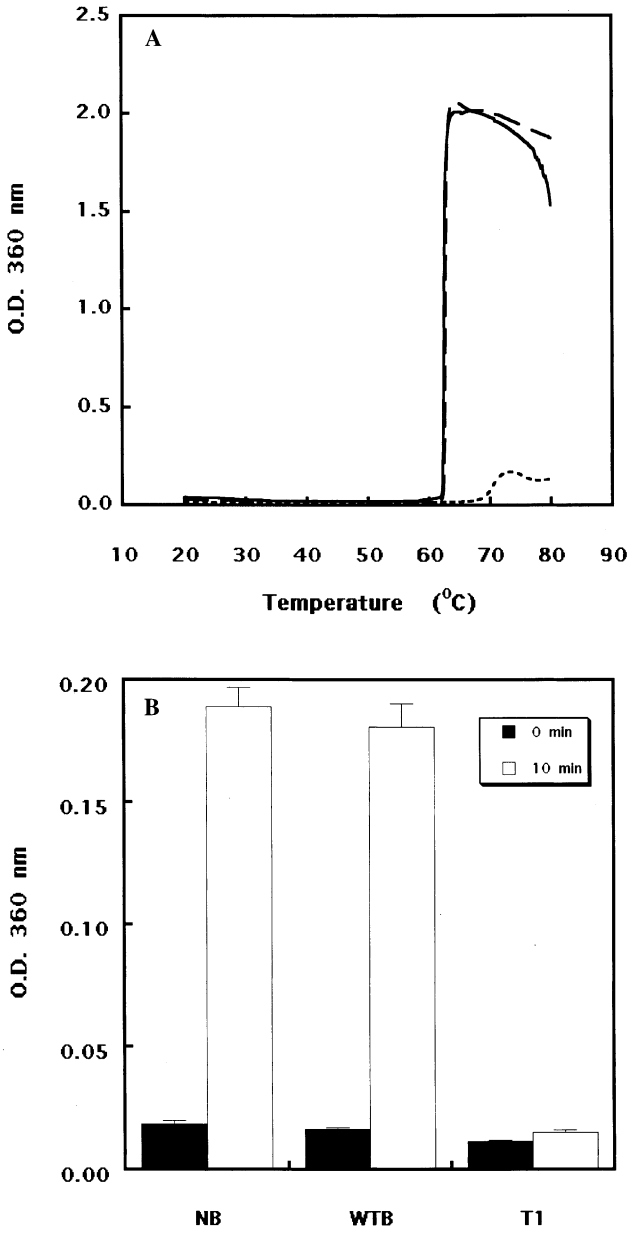

Fig. 4. Comparison of thermal stability for native αB, recombinant WT-αB, and T1 mutant. (A) Native purified αB-crystallin (solid line), recombinant wild-type αB-crystallin (dash line), and T1 (dot line) of identical concentration (36 μM in 0.5 ml) were analyzed for their thermal stability by heating the samples from 20 to 80 °C. Turbidity changes for each crystallin solution were followed continuously by measuring OD (optical density) at 360 nm. (B) The anti-thermal aggregation capabilities of native αB, recombinant wild-type αB, and T1 truncated mutant crystallins were assayed at 70 °C. Samples were incubated at 70 °C for 10 min and the extent of light scattering (OD 360 nm) was measured before (black bars) and after (white bars) incubation. It clearly indicates that T1 lysine truncated mutant is stable at 70 °C.

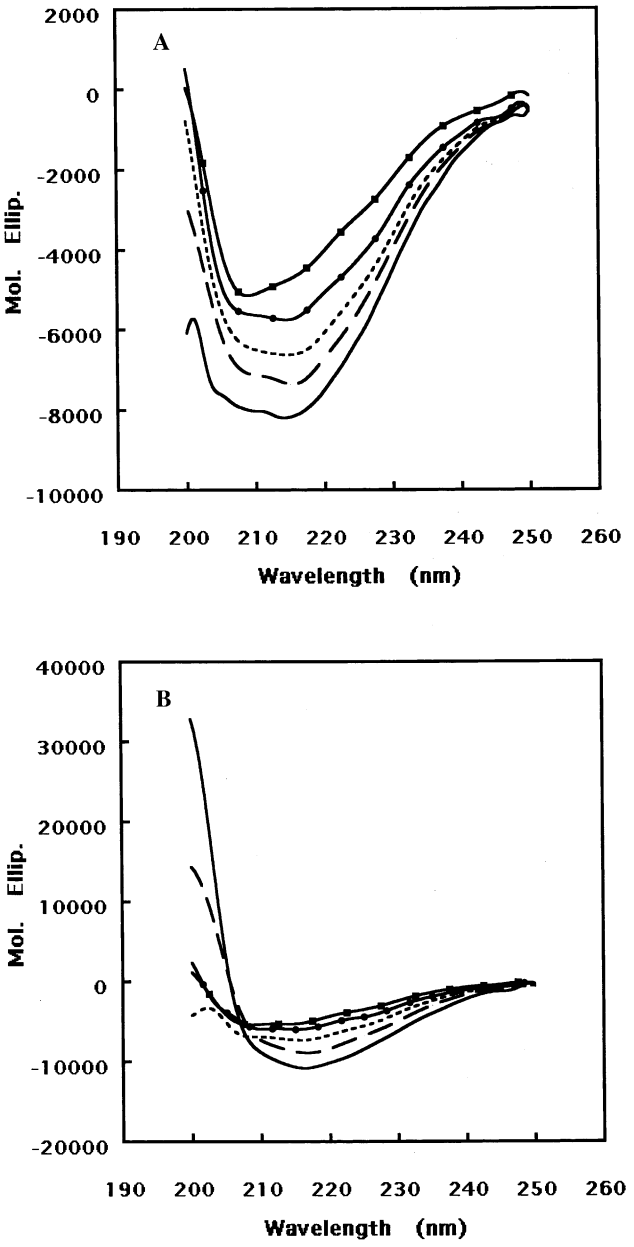

Fig. 5. The CD spectral changes of recombinant wild-type αB and T1 truncated mutant by heating at different temperatures. (A) The CD spectra of wild-type αB-crystallin at 20 °C (solid line with square), 40 °C (solid line with circle), 50 °C (dot line), 55 °C (dash line), and 60 °C (solid line). (B) The CD spectra of T1 at 20 °C (solid line with square), 40 °C (solid line with circle), 50 °C (dot line), 60 °C (dash line), and 70 °C (solid line). Secondary structural estimation based on CD spectra using SELCON3 algorithm program [24] revealed that by increasing temperature from 20 to 60 °C, wild-type αB-crystallin showed an increase in the proportion of unordered structure relative to β-sheet. In contrast, T1 mutant appeared to resist major structural change even at 60–70 °C, attesting to its structural stability at high temperatures.

mutant using identical protein concentration in a temperature range of 20–80 °C. The solutions of native purified and wild-type αB-crystallins become turbid at a temperature of 62 °C (transition temperature for αB-crystallin aggregation) or higher, whereas the solution of T1 remains clear, even after heating up to 70 °C (Fig. 4A). To further investigate the anti-thermal aggregation capability of these proteins, we incubated these proteins at 70 °C for 10 min and then measured the extent of light scattering at 360 nm. For each protein, triplicate samples were incubated at 70 °C and light scattering was measured before and after incubation (Fig. 4B). It is evident that light scattering of native and recombinant αB-crystallins increased sharply after incubation at 70 °C; however, T1 remained clear with very low light scattering.

Conformational change of wild-type αB-crystallin and T1 mutant upon heating

To investigate the structural change of recombinant wild-type αB-crystallin and T1 induced by heating, we have employed circular dichroism to monitor the conformational perturbation at different temperatures. Estimation of the secondary-structure composition of these two crystallins based on CD at different temperatures (Fig. 5) was carried out by SELCON3 algorithm program using the expanded reference sets of denatured proteins [24]. By increasing temperature from 20 to 60 °C, wild-type αB-crystallin showed an increase in the proportion of unordered structure relative to β-sheet. In contrast, T1 mutant appears to resist major structural change even at 60–70 °C, attesting to its structural stability at high temperature.

Conclusion

There is a considerable interest in the functional role of αB-crystallin, especially after it was shown to be expressed in various non-lens tissues and is also found to be overexpressed in some tissues of distinct pathological states, such as retinoblastoma [26] and neurodegenerative diseases [27,28]. It is well known that during aging of the normal lens, α-crystallins undergo extensive post-translational modification, including especially proteolytic cleavage from the C-terminus of the molecule [1]. C-terminal lysine truncated αB-crystallin was also reported to constitute between 10% and 90% of the total intact αB-crystallin for human cataractous lenses [14]. In this study, we focus on the comparison of the structure and chaperone activity of native and recombinant αB-crystallins, plus C-terminal lysine truncated mutant. It is very intriguing to find that C-terminal lysine truncated mutant with higher thermostability actually performs better as a molecular chaperone than the intact αB-crystallin. Previously limited tryptic digestion on intact α-crystallin showed that C-terminal truncated αA-crystallin exhibited a decreased ability to protect proteins from heat-induced aggregation using an in vitro assay [12]. Moreover, mutations to the C-terminal lysines

(Lys-174 and Lys-175) greatly diminished the chaperone-like activity of αB-crystallin [29]. Paradoxically, substitution of Lys174–175 of αB-crystallin with Leu–Leu significantly diminished chaperone activity; however, removal of the last five residues had little effect on chaperone activity [30]. Therefore, both hydrophobic and charge–charge interactions probably contribute to the chaperone-like function of αB-crystallin.

In this study, we clearly demonstrate that T1 lysine truncated mutant (minus Lys-174 and Lys-175) is more stable than αB-crystallin at high temperature. Recently, the crystal structure of wheat sHSP 16.9 was solved, revealing the mode how α-crystallin domain and flanking extensions can assemble into a dodecameric double disk [31]. The ability of the C-terminal extension to build different assemblies stems from a hinge between β9 and β10 strands that allow the angle between the α-crystallin domain and the C-terminal extension to vary by 30°, thus, providing some mobile flexibility of C-terminal segment. Such a hinge mechanism may contribute to the size polydispersity observed in the assemblies of most sHSPs and α-crystallin subunits. The elimination of two positive Lys–Lys residues from the C-terminal segment may decrease charge–charge interaction of C-terminal coil with other parts of αB-crystallin, thus, increasing the adaptability of C-terminal extension to bind the unfolded substrate polypeptides and promoting chaperone activity. We have recently demonstrated that both surface hydrophobicity and structural stability play some roles in the molecular mechanism underlying the chaperone-like activity of α-crystallin and its subunit aggregates [32]. As a member of small heat-shock proteins, αB-crystallin may be a better chaperone than αA-crystallin under most thermal stress. The creation of C-terminal truncated mutants with high thermostability and anti-thermal aggregation propensity may prove to be a better molecular chaperone suitable for detailed functional characterization.

Acknowledgments

This work was supported in part by Academia and the National Science Council (NSC Grants 87-2311-B-002-068, 88-2311-B-002-061, and 89-2311-B-001-190 to S.-H. Chiou), Taipei, Taiwan. This report will be submitted as part of a dissertation by J.-H. Liao to National Taiwan University in partial fulfillment of the degree of Doctor of Philosophy. We thank Professor Gu-Gang Chang at the Department of Biochemistry, National Defense Medical Center, Taiwan, for assisting sedimentation velocity and equilibrium analyses.

References

[1] J.J. Harding, K.J. Dilley, Structural proteins of the mammalian lens: a review with emphasis on changes in development, aging and cataract, Exp. Eye Res. 22 (1976) 1–73.

[2] T.D. Ingolia, E.A. Craig, Four small Drosophila heat shock proteins are related to each other and to mammalian α-crystallin, Proc. Natl. Acad. Sci. USA 79 (1982) 2360–2364.

[3] J. Horwitz, α-Crystallin can function as a molecular chaperone, Proc. Natl. Acad. Sci. USA 89 (1992) 10449–10453.

[4] R.A. Dubin, E.F. Wawrousek, J. Piatigorsky, Expression of the murine αB-crystallin gene is not restricted to the lens, Mol. Cell. Biol. 9 (1989) 1083–1091.

[5] S. Dasgupta, T.C. Hohman, D. Carper, Hypertonic stress induces αB-crystallin expression, Exp. Eye Res. 54 (1992) 461–470.

[6] W.W. de Jong, J.A. Leunissen, C.E. Voorter, Evolution of the α-crystallin/small heat-shock protein family, Mol. Biol. Evol. 10 (1993) 103–126.

[7] L.R. Lin, D. Carper, T. Yokoyama, V.N. Reddy, The effect of hypertonicity on aldose reductase, αB-crystallin, and organic osmolytes in the retinal pigment epithelium, Invest. Ophthalmol. Vis. Sci. 34 (1993) 2352–2359.

[8] G.J. Caspers, J.A. Leunissen, W.W. de Jong, The expanding small heat-shock protein family, and structure predictions of the conserved ''α-crystallin domain'', J. Mol. Evol. 40 (1995) 238–248.

[9] C.J. Bult, O. White, G.J. Olsen, L. Zhou, R.D. Fleischmann, G.G. Sutton, J.A. Blake, L.M. FitzGerald, R.A. Clayton, J.D. Gocayne, A.R. Kerlavage, B.A. Dougherty, J.F. Tomb, M.D. Adams, C.I. Reich, R. Overbeek, E.F. Kirkness, K.G. Weinstock, J.M. Merrick, A. Glodek, J.L. Scott, N.S.M. Geoghagen, J.C. Venter, Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*, Science 273 (1996) 1058–1073.

[10] R.H. Russnak, D. Jones, E.P. Candido, Cloning and analysis of cDNA sequences coding for two 16 kDa heat shock proteins (hsps) in *Caenorhabditis elegans*: homology with the small hsps of Drosophila, Nucleic Acids Res. 11 (1983) 3187–3205.

[11] G. Wistow, Domain structure and evolution in α-crystallins and small heat-shock proteins, FEBS Lett. 181 (1985) 1–6.

[12] L. Takemoto, T. Emmons, J. Horwitz, The C-terminal region of α-crystallin: involvement in protection against heat-induced denaturation, Biochem. J. 294 (1993) 435–438.

[13] J.A. Carver, J.A. Aquilina, R.J. Truscott, G.B. Ralston, Identification by ^{1}H NMR spectroscopy of flexible C-terminal extensions in bovine lens α-crystallin, FEBS Lett. 311 (1992) 143–149.

[14] J. Jimenez-Asensio, C.M. Colvis, J.A. Kowalak, Y. Duglas-Tabor, M.B. Datiles, M. Moroni, U. Mura, C.M. Rao, D. Balasubramanian, A. Janjani, D. Garland, An atypical form of αB-crystallin is present in high concentration in some human cataractous lenses. Identification and characterization of aberrant N- and C-terminal processing, J. Biol. Chem. 274 (1999) 32287–32294.

[15] L.J. Takemoto, Changes in the C-terminal region of αA-crystallin during human cataractogenesis, Int. J. Biochem. Cell Biol. 29 (1997) 311–315.

[16] P. Lin, D.L. Smith, J.B. Smith, In vivo modification of the C-terminal lysine of human lens αB-crystallin, Exp. Eye Res. 65 (1997) 673–680.

[17] M. Cherian, E.C. Abraham, Decreased molecular chaperone property of α-crystallins due to posttranslational modifications, Biochem. Biophys. Res. Commun. 208 (1995) 675–679.

[18] J.A. Carver, K.A. Nicholls, J.A. Aquilina, R.J. Truscott, Age-related changes in bovine α-crystallin and high-molecular-weight protein, Exp. Eye Res. 63 (1996) 639–647.

[19] R. Smulders, J.A. Carver, R.A. Lindner, M.A. van Boekel, H. Bloemendal, W.W. de Jong, Immobilization of the C-terminal extension of bovine αA-crystallin reduces chaperone-like activity, J. Biol. Chem. 271 (1996) 29060–29066.

[20] R.A. Lindner, J.A. Carver, M. Ehrnsperger, J. Buchner, G. Esposito, J. Behlke, G. Lutsch, A. Kotlyarov, M. Gaestel, Mouse Hsp25, a small heat-shock protein. The role of its C-terminal

extension in oligomerization and chaperone action, Eur. J. Biochem. 267 (2000) 1923–1932.
[21] J.-H. Liao, C.-C. Hung, J.-S. Lee, S.-H. Wu, S.-H. Chiou, Characterization, cloning, and expression of porcine αB-crystallin, Biochem. Biophys. Res. Commun. 244 (1998) 131–137.
[22] S.C. Gill, P.H. von Hippel, Calculation of protein extinction coefficients from amino acid sequence data, Anal. Biochem. 182 (1989) 319–326.
[23] P. Schuck, Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling, Biophys. J. 78 (2000) 1606–1619.
[24] N. Sreerama, R.W. Woody, Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set, Anal. Biochem. 287 (2000) 252–260.
[25] M. Bloemendal, A. Toumadje, W.C. Johnson Jr., Bovine lens crystallins do contain helical structure: a circular dichroism study, Biochim. Biophys. Acta 1432 (1999) 234–238.
[26] R.D. Pineda, C.C. Chan, M. Ni, B.J. Hayden, M.A. Johnson, J. Nickerson, G.J. Chader, Human retinoblastoma cells express αB-crystallin in vivo and in vitro, Curr. Eye Res. 12 (1993) 239–245.
[27] S. Kato, A. Hirano, T. Umahara, M. Kato, F. Herz, E. Ohama, Comparative immunohistochemical study on the expression of αB-crystallin, ubiquitin and stress-response protein 27 in ballooned neurons in various disorders, Neuropathol. Appl. Neurobiol. 18 (1992) 335–340.
[28] J. Lowe, D.R. Errington, G. Lennox, I. Pike, I. Spendlove, M. Landon, R.J. Mayer, Ballooned neurons in several neurodegenerative diseases and stroke contain αB-crystallin, Neuropathol. Appl. Neurobiol. 18 (1992) 341–350.
[29] M.L. Plater, D. Goode, M.J.C. Crabbe, Effects of site-directed mutations on the chaperone-like activity of αB-crystallin, J. Biol. Chem. 271 (1996) 28558–28566.
[30] J.A. Carver, N. Guerreiro, K.A. Nicholls, R.J.W. Truscott, On the interaction of α-crystallin with unfolded proteins, Biochim. Biophys. Acta 1252 (1995) 251–260.
[31] R.L. van Montfort, E. Basha, K.L. Friedrich, C. Slingsby, E. Vierling, Crystal structure and assembly of a eukaryotic small heat shock protein, Nat. Struct. Biol. 8 (2001) 1025–1030.
[32] J.-H. Liao, J.-S. Lee, S.-H. Chiou, Distinct roles of αA- and αB-crystallins under thermal and UV stresses, Biochem. Biophys. Res. Commun. 295 (2002) 854–861.