Volume 236, number 1, 109–114 FEB 06173 August 1988

Heat-induced changes in the conformation of α- and β-crystallins: unique thermal stability of α-crystallin

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Received 6 May 1988; revised version received 20 June 1988

Of the crystallin proteins of the lens, the principal subunit of the β-crystallin, βB2 (βBp), has been considered to be the only heat-stable protein because it does not precipitate upon heating. In our recent investigations, however, we have found that the α-crystallin from bovine lenses is not only heat stable but also does not denature at temperatures up to 100°C. Using circular dichroism and fluorescence to monitor the conformational changes of α- and βB2-crystallins upon heating, we found that α-crystallin maintains a high degree of structure, whereas the βB2-crystallin shows a reversible sigmoidal order-disorder transition at about 58°C.

α-Crystallin; β-Crystallin; Thermal stability; Conformational change

1. INTRODUCTION

Among the cytoplasmic soluble protein components (α-, β-, γ-crystallins) of the mammalian lens fiber cells, α-crystallin is the largest [1]. It is an oligomeric, globular protein of $M_r \sim 8 \times 10^5$ and is composed of two gene-product subunits, α_A and α_B, each with an M_r of $\sim 2 \times 10^4$. The β-crystallin is also a multimer, unlike the γ-crystallin which is monomeric. During aging and cataractogenesis, considerable changes in the crystallins have been noted, including many involving α-crystallin [1–3]. Yet this protein, particularly in humans, survives longer than the other two during aging [4].

The stability of lens proteins is of particular importance because they cease turning over shortly after their synthesis, unlike most other proteins. We have, thus, undertaken a systematic investigation [5–13] of the structure and stability of the lens crystallins. These studies were aimed at assessing changes in these molecules resulting from the normal aging process and cataractogenesis.

Recently, investigators [14–16] have focused their attention on the principal subunit of the β-crystallin, the polypeptide βB2 (formerly known as βBp), because it is the only mammalian lens crystallin that does not precipitate when heated to 100°C; this so-called 'thermal stability' has been related [15] to the ability of different species to withstand the environmental temperature conditions. To confirm this property of βB2, we included this subunit in this study.

We used circular dichroism (CD) and fluorescence techniques to monitor the thermal changes of these two proteins. We report here that α under certain conditions is remarkably more stable than βB2 in that the former does not denature even at 100°C. βB2, on the other hand, undergoes a reversible order-disorder transition at about 60°C but does not precipitate at 100°C.

2. MATERIALS AND METHODS

α-, β- and γ-crystallins from calf lenses were initially separated by gel filtration on a Sephacryl S-200 column [17].

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

The α-crystallin was purified further on a Biogel A-15m column [5]. The low-molecular-mass fraction of the α (α_L) from this separation was collected. Cortical and nuclear α_L were indistinguishable in this study. βB2 was isolated from the β_L fraction following the method described [15].

CD studies were conducted using an AVIV 60DS spectropolarimeter (AVIV Associates, Lakewood, NJ) [8] with a temperature control accessory (Hewlett-Packard, Palo Alto, CA) to monitor changes in the ellipticity at 217 nm wavelength as the temperature increased up to 100°C in increments of 0.2–1.0°C. Protein concentrations were 0.1–0.2 mg/ml with a 0.1 or 1.0 cm light path for far-UV CD measurements and 1.0–2.0 mg/ml with a 1.0 cm light path for near-UV measurements [8]. Secondary structure calculations were made by computer analysis of the far-UV CD spectra using the method of Chang et al. [18].

Fluorescence was measured using a Perkin-Elmer MPF 44A fluorometer [10], and the temperature was controlled by a circulating water bath. Sample temperature was determined using a thermocouple inserted into a cuvette.

3. RESULTS

The far-UV CD spectra of both α_L (fig.1A) and βB2 (fig.1B) at 25°C show a minimum around 217 nm, which is characteristic of a β-conformation. As the temperature increases, the minimum for α_L becomes more negative but levels off at 60–100°C. In the lower frame of fig.1A, the change in the CD minimum at 217 nm with respect to temperature is shown schematically. The spectrum of α_L at higher temperatures (60–100°C) reflects more of an α-helical structure than of a random coil. The CD spectrum of βB2, on the other hand, shows a decrease in magnitude (less negative) at 217 nm (fig.1B), and at temperatures above 60°C the spectrum resembles that of a protein with a random-coil structure. A sharp, sigmoidal, order-disorder transition of the structure is seen when the ellipticity at 217 nm is plotted against temperature (fig.1B, lower frame). After heating to 100°C, the α_L and βB2 samples were cooled to room temperature (25°C), and the spectra of both were recorded (fig.1). Both spectra are very similar to that of the unheated one, that is, mostly reversible. Computer analysis of the far-UV CD data of both α_L and βB2 shows that the α-helical structure increases from 4% to 12% upon heating to 100°C in the α_L-crystallin. However, in βB2 this structure becomes more random at temperatures above 60°C.

Fig.2 shows the change in intensity and emission maximum (λ_{max}) of tryptophan fluorescence of both α_L and βB2 as a function of temperature. The λ_{max} for α_L remains unchanged, whereas the λ_{max} for βB2 shifts from 330 to 348 nm around 60°C; these results agree with those from the far-UV CD analysis. Since the free tryptophan emits at 350 nm, the red shift to 348 nm indicates that βB2 becomes a random coil at that temperature and above. The decrease in fluorescence intensity of both proteins upon increasing temperature is probably due to thermal deactivation of the excited state of the molecules.

The thermal stability of α_L-crystallin is dependent on the concentration of protein and the buffer used for solution (fig.3). Aggregation starts when the protein concentration is 0.05 mg/ml in Tris-KCl buffer, pH 7.4, and 2.15 mg/ml in phosphate buffer, pH 7.4.

4. DISCUSSION

Spectroscopic evaluation of the secondary and tertiary structures of α-crystallin has been detailed [5–7,19]; the protein chain consists primarily of the β-sheet conformation with less than 5% of the α-helix structure. The increase in α-helical content upon heating might raise the question as to whether heat induces dissociation (or disaggregation) of the α into αA and αB subunits. If one of the subunits were to contain more of an α-helical structure than the other, upon heating there could be a net increase in α-helical structure during dissociation. Because of the similarities of the sequences [20] and far-UV CD properties of the subunits [21], it is unlikely, however, that αA and αB would differ significantly in their secondary structure. Moreover, the far-UV CD spectrum returns to its original state upon cooling. In view of these facts, heat-induced dissociation of subunits seems improbable. Alternatively, at higher temperatures the quaternary structure may change, as suggested [22], and the monomers may rearrange themselves in a slightly different conformation (with more α-helix than the original). Unlike with heat, α-crystallin completely denatures in urea or guanidine hydrochloride [23].

The heat stability of α-crystallin depends on solvent conditions (buffer) and protein concentration (fig.3). As a result, it appears that the heat-induced precipitation at higher concentrations of protein or under other solvent conditions is not preceded by

denaturation but is a 'super aggregation' process of the α-crystallin multimer.

The unique and remarkable conformational (thermal) stability of α-crystallin observed in this study may provide a new concept in understanding the structure and function of this major protein of the mammalian lens. Various models for the quaternary structure of the α-crystallin aggregate have been proposed, including the three-layer [22,24], dodecamer [25], and micellar [21,26]

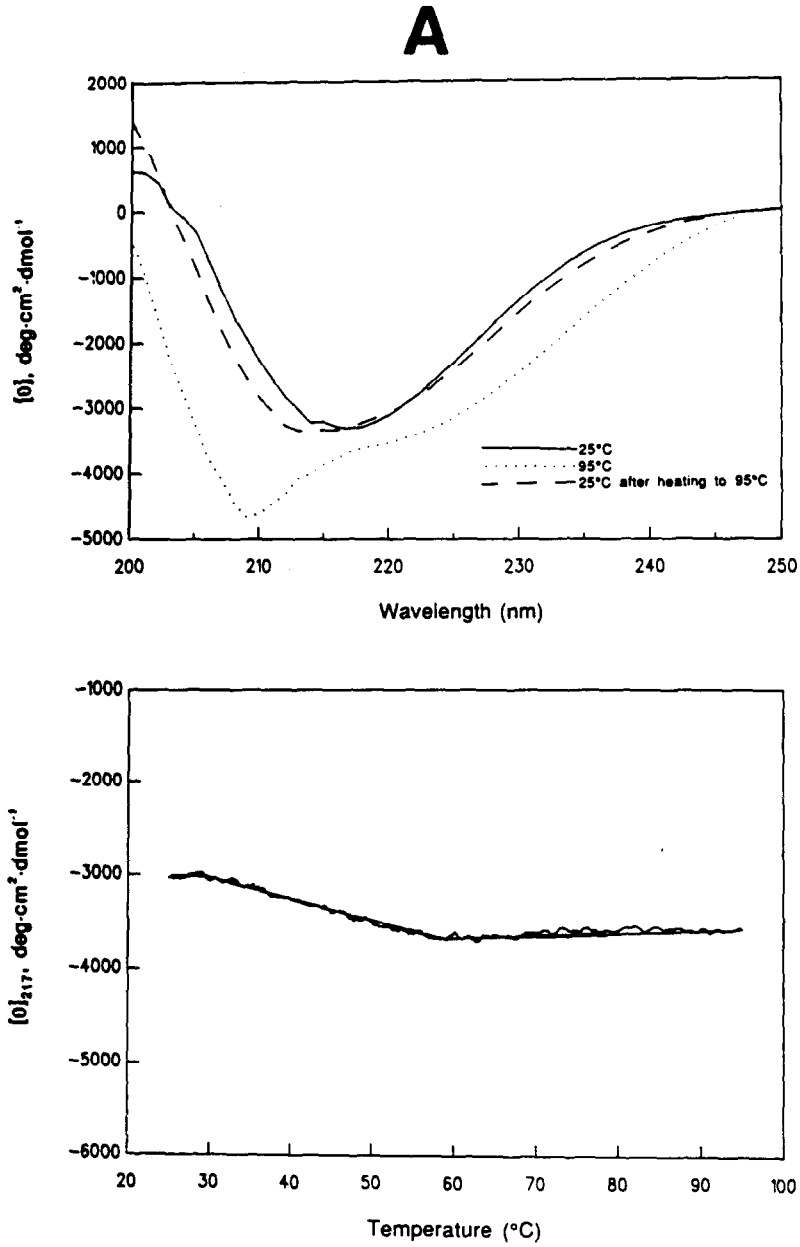
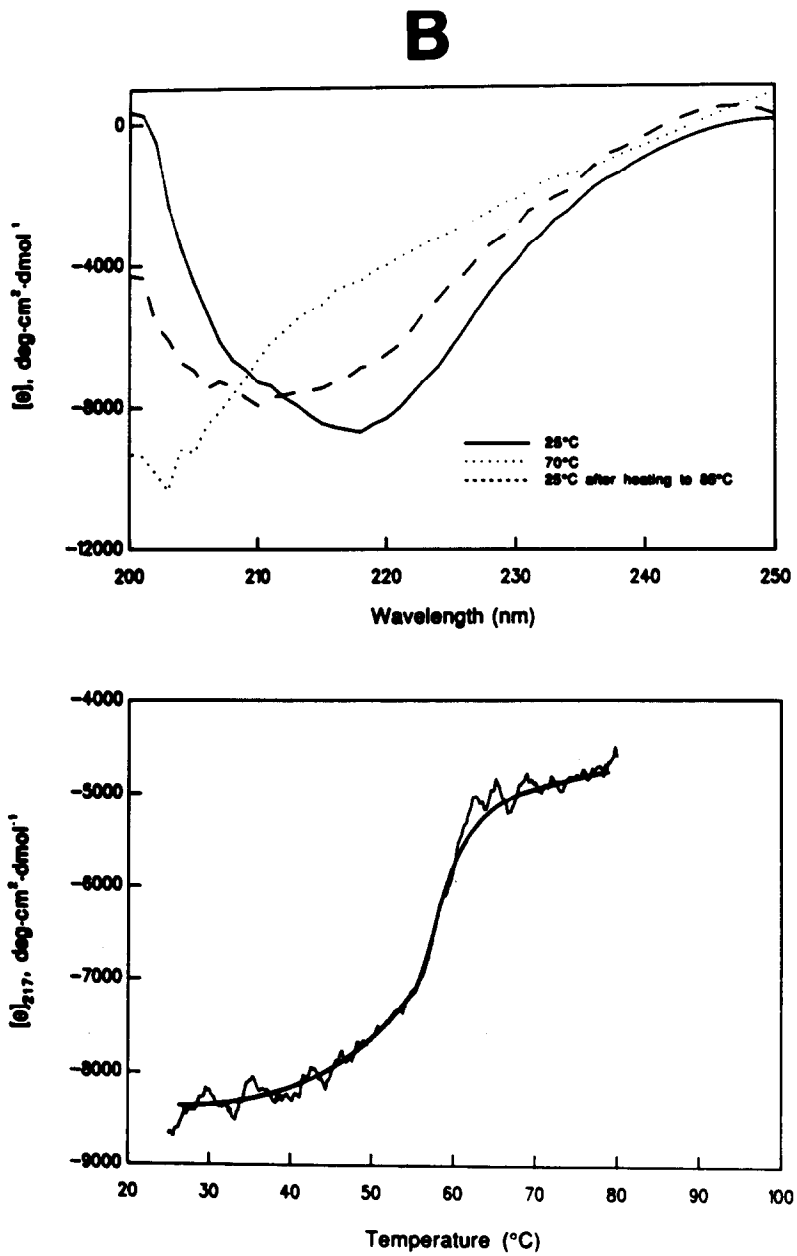


Fig.1. (A) Far-UV CD spectra of α_L at 25°C, 90°C and 25°C after heating to 95°C (upper frame). Change in ellipticity at 217 nm with respect to temperature (lower frame). (B) Far-UV CD spectra of βB2 at 25°C, 70°C and 25°C after heating to 85°C (upper frame). Change in ellipticity at 217 nm with respect to temperature (lower frame). Protein concentration was 0.1 mg/ml in 0.1 M phosphate buffer, pH 7.4, in each case.

structures. Perhaps further study on the temperature-induced structural changes of α-crystallin under a variety of conditions will provide information consistent with one of these suggested models.

It is now evident that βB2 is not conformationally stable at higher temperatures. Reported data on thermal stability of βB2 [14–16] are somewhat misleading in that the protein is indeed denatured above 60°C. However, the denaturation is largely reversible, which has misled previous investigators. In general, the melting temperature, order-disorder transition, and thermal behavior of βB2 resemble those of the γ-crystallins more than those of α-crystallin. The melting temperatures of γ-II, γ-III, and γ-IV are 70.5, 75.2 and 75.4°C, respectively [27], compared with ~58°C for βB2. The order-disorder transition of both γ-crystallins and



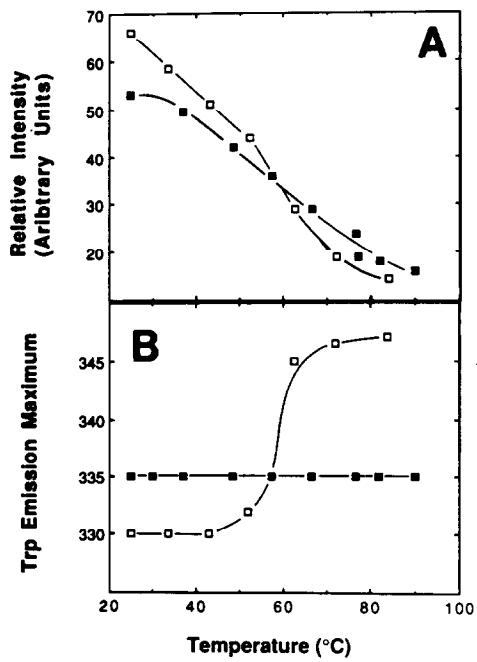


Fig.2. Change in tryptophan (A) fluorescence intensity and (B) emission maximum of α_L (solid squares) and βB2 (open squares) as a function of temperature. Protein concentrations of both were 0.1 mg/ml in 0.1 M phosphate buffer, pH 7.4.

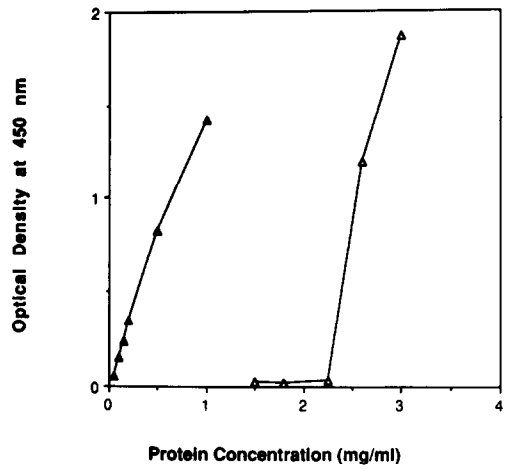


Fig.3. Turbidity measurements of different concentrations of α_L after heating to 100°C for 5 min in 0.1 M phosphate buffer, pH 7.4 (open triangles), and in Tris/KCl buffer, pH 7.4 (solid triangles); the latter buffer contained 0.01 M Tris-HCl, 0.1 M KCl, 0.0008 M EDTA, 0.003 M sodium azide and 0.0007 M 2-mercaptoethanol. The degree of turbidity was determined by measuring the absorbance at 450 nm.

βB2 is sharp and sigmoidal. The only similarity of βB2 to the α-crystallin is that the temperature-induced conformational change is reversible; no precipitation occurs upon cooling to room temperature. γ-Crystallins, on the other hand, form aggregates at temperatures higher than their melting temperature [27]. βB2 shows 29% sequence homology with bovine γ-II [28]. X-ray studies [29–31] also suggest that a structural similarity exists between βB2 and γ-II crystallin. The major difference between γ-II and βB2, however, is that the structure of the latter favors formation of dimers [32]. Despite this difference, studies [28,31] including this report strongly favor the concept of a β-γ superfamily with respect to resemblance in structure and in properties.

There are currently many investigations aimed at determining the exact structures of the crystallins and how they may change with time. The α-crystallin, for example, may increase in molecular mass with age but maintain the same secondary structure [33], become insoluble [1–3]. The specific mechanisms for these and other changes are not always known. Stability studies such as this one will continue to shed light on the structure of the lens proteins and provide explanations for the resilience of the lens with aging. The ability of the α-crystallins when heated to maintain an ordered secondary structure through an accommodative process is indeed unique among the crystallins. Our findings confirm the speculated thermodynamic stability of the α-crystallin because of its sequence homology with heat shock proteins [34]. Ironically, recent studies on the thermal stability of crystallins have centered on the βB2 which denatures. To understand better the thermal behavior of the α-crystallin, future studies could focus on the contributions and arrangement of its subunits or the effect of protein modification on the thermal stability of this crystallin.

Acknowledgement: This study was supported by NIH grant EY04161.

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