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Thermal stability and reversibility of secondary conformation of α-crystallin membrane during repeated heating processes

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Abstract

Reflectance FT-IR/DSC microspectroscopy was first used to study the structural conformation of α-crystallin membranes in the heating–cooling–reheating cycle. The thermotropic transition and the changes in secondary structure of α-crystallin membrane during heating and reheating processes were investigated. A thermal transition ranging between 50 and 70°C with a midpoint at 60°C for the α-crystallin membrane was easily obtained from the three-dimensional plots of the reflectance FT-IR spectra as a function of temperature. The secondary structural components of the α-crystallin membrane were modified step-by-step with the increase of temperature from 25 to 120°C, but restored to original values after cooling to 25°C. During the heating process, the compositions of the α-helix, random coil and β-sheet structure decreased with temperature, but the content of the β-turn structure increased, however, all of them were restored after cooling. The absence of significant alteration in the secondary structures for the α-crystallin membrane before and after the first-heating process strongly suggests the high thermal stability and reversibility of α-crystallin. Interestingly, the thermal behavior of the first-heated α-crystallin membrane during the reheating process exhibited a unique thermal behavior with two transitional temperatures at 35–50 and 55–70°C. The reflectance FT-IR/DSC microscopic data indicated that α-crystallin in the membrane state had higher thermal stability and reversibility.

Keywords: α-Crystallin; Membrane; Secondary structure; Thermotropic transition; Reflectance FT-IR/DSC microspectroscopy; Thermal stability; Reversibility

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1. Introduction

α-Crystallin is not only acknowledged as a lens-specific protein but also found in many other cells and organs [1–3]. Many studies have revealed that α-crystallin exhibits a chaperone-like activity to prevent the loss of enzyme activity, and the insolubilization as well as the aggregation of other proteins [3–6]. The chaperone-like activity of α-crystallin has been studied to suppress the cataract formation induced by calpain II in vitro or selenite in vivo, due to the interaction with aged/damaged proteins in lens [7,8]. The molecular mechanism of the chaperone action of α-crystallin is still unknown, but the inclusion of enzyme or protein into the central cavity of α-crystallin (Carver's model) [9], and/or the exposure of the hydrophobic surfaces of α-crystallin have been proposed [10,11]. Recent studies [8,10,11] have also proposed that temperature-induced exposure of hydrophobic surfaces of α-crystallin conformation might enhance the chaperone activity of α-crystallin. A structural transition above 30°C has been evidenced to induce the formation of a hydrophobic surface and to enhance chaperone activity, but another study has showed that at temperature above 38°C the protein became irreversible in transition [10]. Although the intact transitional temperature is still divergent, temperature no doubt plays an important role to effect the chaperone-like activity of α-crystallin.

There are a number of divergent results concerning the heat-induced conformational stability of α-crystallin in the liquid state. It has been reported that α-crystallin was not only heat stable but also did not denature until beyond 100°C, and that there was no thermal transition below 100°C by using circular dichroism [12]. On the other hand, some have indicated a thermal transition of α-crystallin within 60–75°C by calorimetric and NMR measurements [13–16]. However, the conformational stability of α-crystallin has also been reported to be rather low due to the lower free energy at the thermal transition [16]. α-Crystallin in D_2O has been investigated to show a thermotropic transition temperature at 60–62°C, but its infrared spectroscopic result also indicated a massive loss of its secondary structure with the increase in temperature [17]. Those uncertain thermal behaviors of α-crystallin are suspicious, and it is worth attempting to make sure whether the α-crystallin has intrinsic thermal stability and reversibility even by using another analytical method. In this study, a membrane sample of α-crystallin was prepared instead of a liquid sample.

A novel microscopic Fourier transform infrared spectroscope equipped with differential scanning calorimetry (FT-IR/DSC system) has been used in our previous study to simultaneously determine the correlation between the thermal response and the structural change of drugs, polymers and skin [18–22]. The operation of this system is fast, simple, sensitive, precise and reproducible. In the present study, we also used this microscopic FT-IR/DSC system with reflectance mode to examine the thermal stability and conformational structure of the α-crystallin membrane in a heating–cooling–reheating cycle. The thermal reversibility of α-crystallin conformation was also explored. Our results indicate that the protein secondary conformation of α-crystallin even in the membrane state had high thermal stability and reversibility.

2. Materials and methods

2.1. Materials

α-Crystallin isolated from bovine eye lens was purchased from Sigma Chem. Co. (St. Louis, MO, USA). The protein concentration was approx. 88% determined by the biuret method.

2.2. Preparation of the α-crystallin membrane

One drop of 0.125% α-crystallin aqueous solution was dropped onto the aluminum foil (5 mm^2). A spin coater (SC-300, E.H.C. Co., Taiwan) was used to spread this droplet [21,22], and stored at 25°C, 50% RH condition. After storage for 1 day, water was evaporated and α-crystallin membrane casted on the foil was formed.

2.3. Reflectance FT-IR / DSC microscopic measurements

The sample of α-crystallin membrane on the foil was put directly into the DSC microscopy cell (FP 84, Mettler, Switzerland). This cell was then settled on the stage of the FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Japan) with an MCT (mercury-cadmium telluride) detector. The system was operated in the reflectance mode. The position and focus of the sample were adjusted microscopically. The desired sample size (related to sample concentration) for determination was selected and defined by means of an Aperture Through Optical System (ATOS) for analysis. The temperature of the DSC system was monitored with a central processor (FP 80HT, Mettler, Switzerland). The heating rate of the DSC assembly was controlled at 3°C/min. The reflectance FT-IR/DSC system was carried out with a non-isothermal method using a heating program from 25 to 120°C for first heating and 25–80°C for the second heating. The DSC heating program and IR spectra could be simultaneously recorded [18–22]. The reflectance IR spectra were collected at an angle of incidence centered at 30° taken with a resolution of 4 cm^{-1}. Several spectra of the sample at pre-described heating temperatures were individually picked up and the H_2O combination band near 2120–2150 cm^{-1} subtracted, due to the combination of OH stretching and H–O–H bending vibrations of the residual water [23]. The composition of each component in the amide I band of these spectra were estimated quantitatively by a curve fitting program with the minimum standard error. The program iterates the curve-fitting process according to the mix (Gaussian–Lorenzian) function using a full-bandwidth of 10 cm^{-1} and a resolution enhancement factor of 1.5. The proportion of a component was computed to be the fractional area of the corresponding peak, divided by the sum of the areas of all the peaks.

3. Results

Fig. 1 shows the three-dimensional plots of the reflectance FT-IR spectra of α-crystallin membrane between 1720 and 1000 cm^{-1}, as a function of temperature. The amide I band was found to have one maximum peak at 1635 cm^{-1} assigned to the β-sheet structure and a small shoulder at 1651 cm^{-1} attributed to the α-helix structure, suggesting a higher proportion of β-sheet conformation in the α-crystallin membrane [23,24]. During the first-heating process, the frequency and shape of the conformation-sensitive amide I band of the α-crystallin membrane did not change with the increase of temperature, but a thermal transition between 50 and 70°C appeared clearly. However, several changes in maximum peak frequency and its intensity of the α-crystallin membrane were evidenced between 1600 and 1000 cm^{-1}. The maximum peaks at 1541, 1394, 1242, 1172 and 1084 cm^{-1} shifted to 1518, 1387, 1223, 1124 and 1035 cm^{-1}, respectively, with the increase of temperature. The peak at 1541 cm^{-1} is assigned to the α-helix structure, and the peak at 1394 cm^{-1} is due to the amide C–N stretching vibration and/or symmetric stretching vibration in carboxylate groups of amino acid side chains [25]. The peak at 1242 cm^{-1} is attributed to the mixture of random coil and β-sheet structures, while the peak at 1172 cm^{-1} is assigned to the contributions of leucine, lysine, tyrosine and valine. The peak at 1084 cm^{-1} is mainly due to the symmetric phosphate stretching mode [25,26]. Fig. 2A reveals the temperature-induced alterations in peak intensity of five specified peaks (1670, 1651, 1643, 1635 and 1620 cm^{-1}) in the amide I band. The peak at 1670 cm^{-1} represents the β-turn structure, but the peaks at 1643 and 1620 cm^{-1} are assigned to random coil and β-sheet structures, respectively. The intensities of all these specified peaks kept constant from 25 to 45–50°C, then markedly decreased to 70°C and finally maintained an almost constant level beyond 70°C. The midpoint of this drastic changed region (50–70°C) was found near 60°C, attributable to the thermotropic transition temperature of the native α-crystallin membrane. This indicates that the reflectance FT-IR/DSC microscopic system can easily and directly determine the transitional temperature of the α-crystallin membrane.

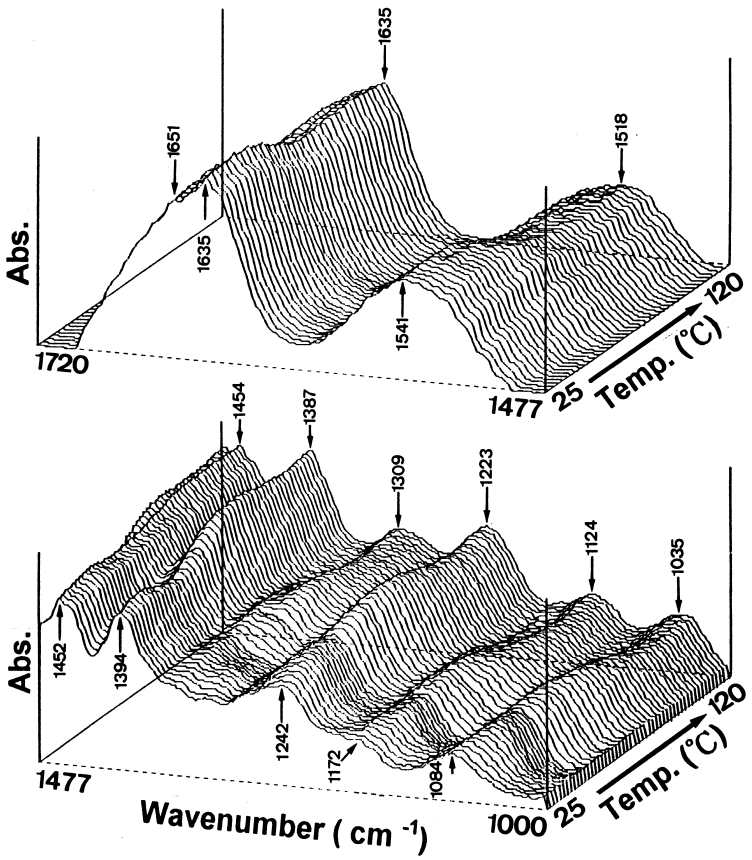

Fig. 1. Three-dimensional plots of reflectance IR spectra of the α-crystallin membrane between 1720 and 1000 cm^{-1} with respect to the first-heating process.

When the first-heated α-crystallin membrane (preheating to 120°C and then cooling to 25°C) was reheated, its three-dimensional reflectance FT-IR spectra between 1720 and 1000 cm^{-1} exhibited a somewhat different fashion and behavior from the plot of Fig. 1, as shown in Fig. 3. Two transitional changes at 35–50 and 55–70°C in the amide I band contour were found for the reheated sample. Moreover, the thermal-induced changes in the region between 1180 and 1000 cm^{-1} seemed to be more pronounced for the reheated sample than the first-heated sample. These two thermal transitions were more obvious in the peak intensity–temperature plot for the reheated sample, as shown in Fig. 2B. The thermal transition at 55–70°C with a midpoint near 62°C was slightly higher than the midpoint of the first-heated sample. The appearance of another transitional change at 35–50°C is unclear, but it seems to be related to the partial dissociation of the α-crystallin membrane structure during the reheating process [27]. Fig. 2 also clearly shows that the intensity values of five specified peaks in the amide I band for the first-heated α-crystallin sample were almost the same as the intensity values of the native α-crystallin membrane without heating. Moreover, the IR spectra of the native α-crystallin membrane and the first-heated α-crystallin membrane almost completely overlapped between 1700 and 1180 cm^{-1}, except the 1180–1000 cm^{-1} region (Fig. 4). The restoration of structural conformation of the first-heated α-crystallin membrane after cooling may be responsible for this result, suggesting that the protein

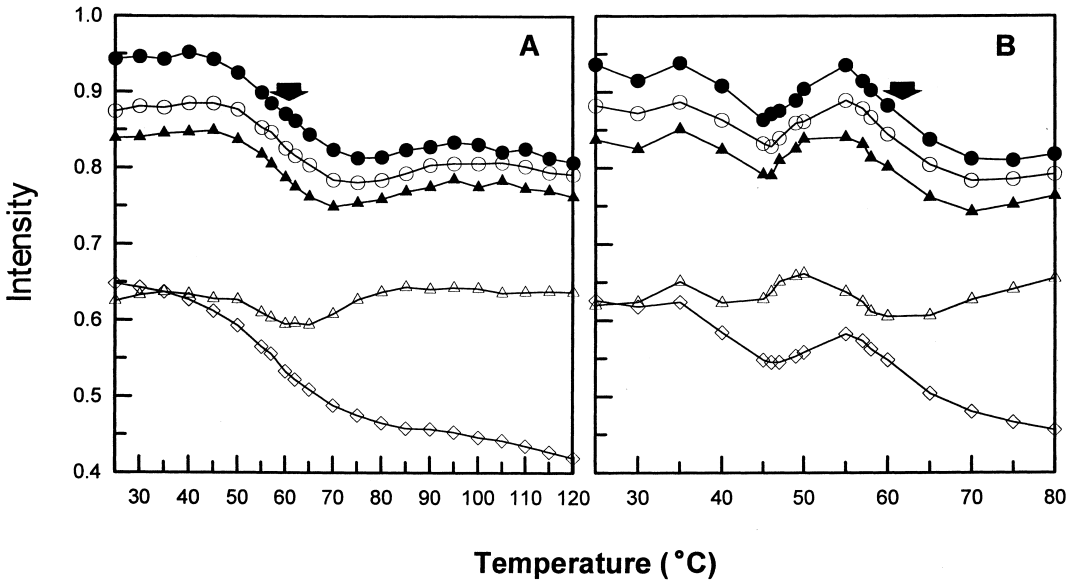

Fig. 2. Temperature-dependent changes for five specified peak intensities in the amide I band of the α-crystallin membrane. Key: A, first heating; B, reheating; ◇, 1620 cm^{-1}; ●, 1635 cm^{-1}; ○, 1643 cm^{-1}; ▲, 1651 cm^{-1}; △, 1670 cm^{-1}.

secondary conformation of the α-crystallin membrane has a higher thermal stability and reversibility.

4. Discussion

α-Crystallin is the major lens structural protein mass in mammalian lenses, and is believed to be related with the maintenance of the transparency and refractive properties of the eyes [3,28]. The chaperone-like activity of α-crystallin has been reported as capable of preventing heat-induced aggregation of various proteins including enzymes, β- and γ-crystallins [3–6], but this activity is age-dependent [29]. Increased amounts of specific modification of α-crystallin may correlate with the change in possible functional role of the α-crystallin molecule [30]. Recent studies indicated that α-crystallin might age-dependently interact with lens membranes and probably result in cataract formation [31–33]. Moreover, the appearance of an increase of $\alpha\beta$-crystallin outside the lens has been associated with various stress-related neuro-degenerative diseases [34,35]. Thus, α-crystallin should play an important role in normal lens and cell functions.

Although several divergent results concerning the heat-induced conformational stability of α-crystallin in the liquid state have been reported [12–17], the present study found that α-crystallin in the membrane state had high thermal stability and reversibility via heating and cooling processes. The conformation-related frequencies of amides I (1700–1590 cm^{-1}), II (1590–1480 cm^{-1}), III (1350–1180 cm^{-1}), the scissoring band of CH_2 and CH_3 (1480–1430 cm^{-1}) and symmetric carboxylate of amino acid side chains (1430–1350 cm^{-1}) for the first-heated α-crystallin membrane at 120°C (Fig. 4c) almost completely overlapped with the frequencies for the native α-crystallin membrane at 25°C (Fig. 4a), although they were somewhat different in the 1180–1000 cm^{-1} region. Furthermore, the thermal-dependent differences in secondary conformational components of the amide I band for the above two samples were not observed, as indicated in Fig. 5 and Table 1. Fig. 5 shows the fitted component bands in the amide I spectrum, in which the peaks at 1691, 1682, 1674, 1667 and 1660 (1659) cm^{-1} are assigned to β-turn structures, the peaks at 1651 and 1643 cm^{-1} are due to the α-helix and random coil conformations, and the peaks at 1635

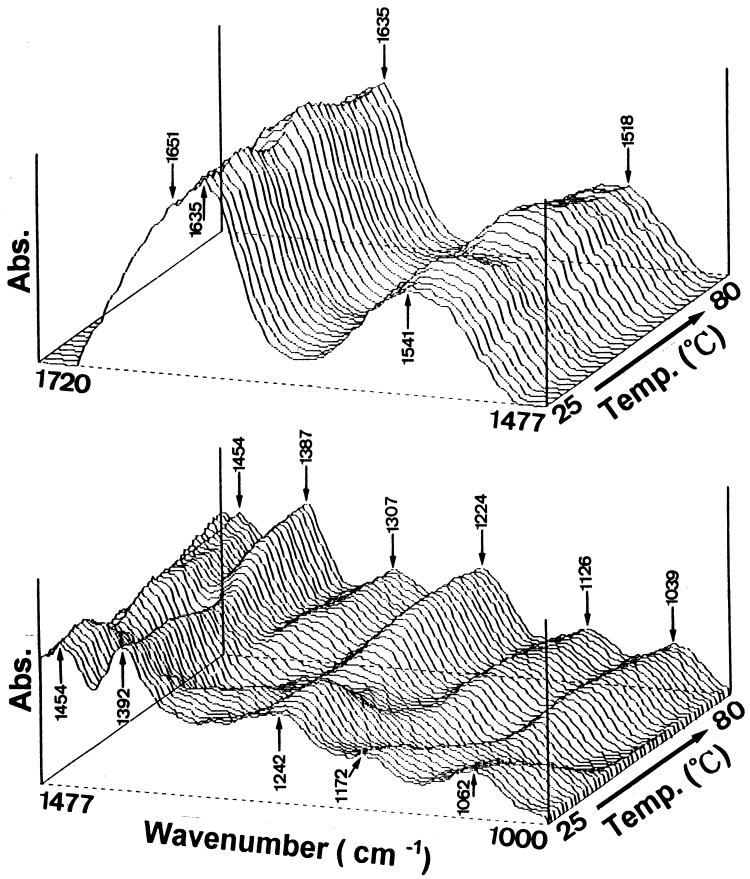

Fig. 3. Three-dimensional plots of reflectance IR spectra of the α-crystallin membrane between 1720 and 1000 cm^{-1} with respect to the reheating process.

(1636), 1627 (1628), 1619 and 1611 cm^{-1} correspond to β-sheet structures [23,26,36,37], respectively. The quantitative estimation of the secondary structure of the α-crystallin membrane before and after heating treatments is listed in Table 1. Obviously, when the α-crystallin membrane was heated from 25 to 120°C, its components of secondary structures changed from 33.71 to 44.70% for the β-turn, from 12.70 to 9.35% for the α-helix, from 13.28 to 10.29% for the random coil, and from 40.31 to 35.66% for β-sheet structures. Once the temperature of the heated sample declined from 120 to 25°C, the components reversibly restored from 44.70 to 33.75% for β-turn, from 9.35 to 12.20% for the α-helix, from 10.29 to 13.07% for the random coil, and from 35.66 to 40.97% for β-sheet structures, near the components of native α-crystallin at 25°C. It also reveals that during the heating process the compositions of the α-helix, random coil as well as β-sheet structure decreased and the content of the β-turn structure increased with temperature, but all of them restored after cooling (Table 1). The absence of significant change in the secondary structures of the α-crystallin membrane before and after the first-heating process strongly suggests that the protein secondary conformation of α-crystallin in the membrane state had high thermal stability and reversibility. During the second-heating process, similar behavior was also found.

The present study easily shows that α-crystallin membrane during first-heating had a thermotropic transition within 50–70°C in which mid-

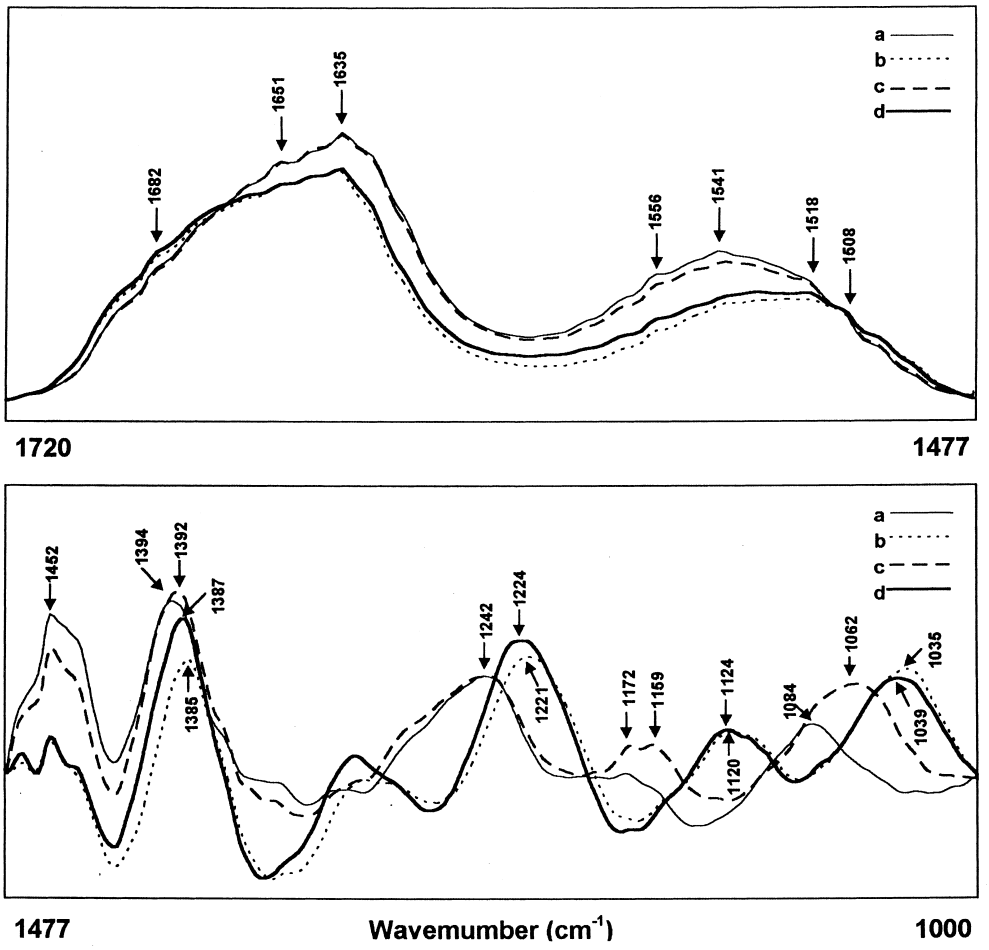

Fig. 4. Comparisons of reflectance IR spectra of the α-crystallin membrane after different heating treatments. Key: a, native α-crystallin membrane without heating (25°C); b, native α-crystallin membrane after first-heating to 120°C; c, b sample after cooling to 25°C; d, c sample after reheating to 80°C.

point was near 60°C (Figs. 1 and 2A), consistent with other results in the liquid state [16,17]. In other words, the reflectance FT-IR/DSC microscopic determination is a very fast, simple, sensitive and precise analytical technique, and the α-crystallin in the membrane state is a suitable model for determination. Evidently the components of the protein secondary structure of the α-crystallin membrane at 60°C were ranged within the components of the protein secondary structure of the α-crystallin membrane at 25 and 120°C, as shown in Table 1, so that the secondary structure of α-crystallin membrane might change progressively during the first-heating process (Figs. 1 and 4) but restore to the original structure after cooling.

The reheated α-crystallin sample produced two thermal transitions at 35–50 and 55–70°C, as shown in the plot of Fig. 2B and Fig. 3. The reason for the transitional change at 35–50°C remains unknown, but it seems to be related to the dissociation of part of the α-crystallin membrane structure [27]. Several studies have proposed that α-crystallin has a three-layer structure assembled by micellar subunits [27,38–40], although this has caused controversy [41]. The in-

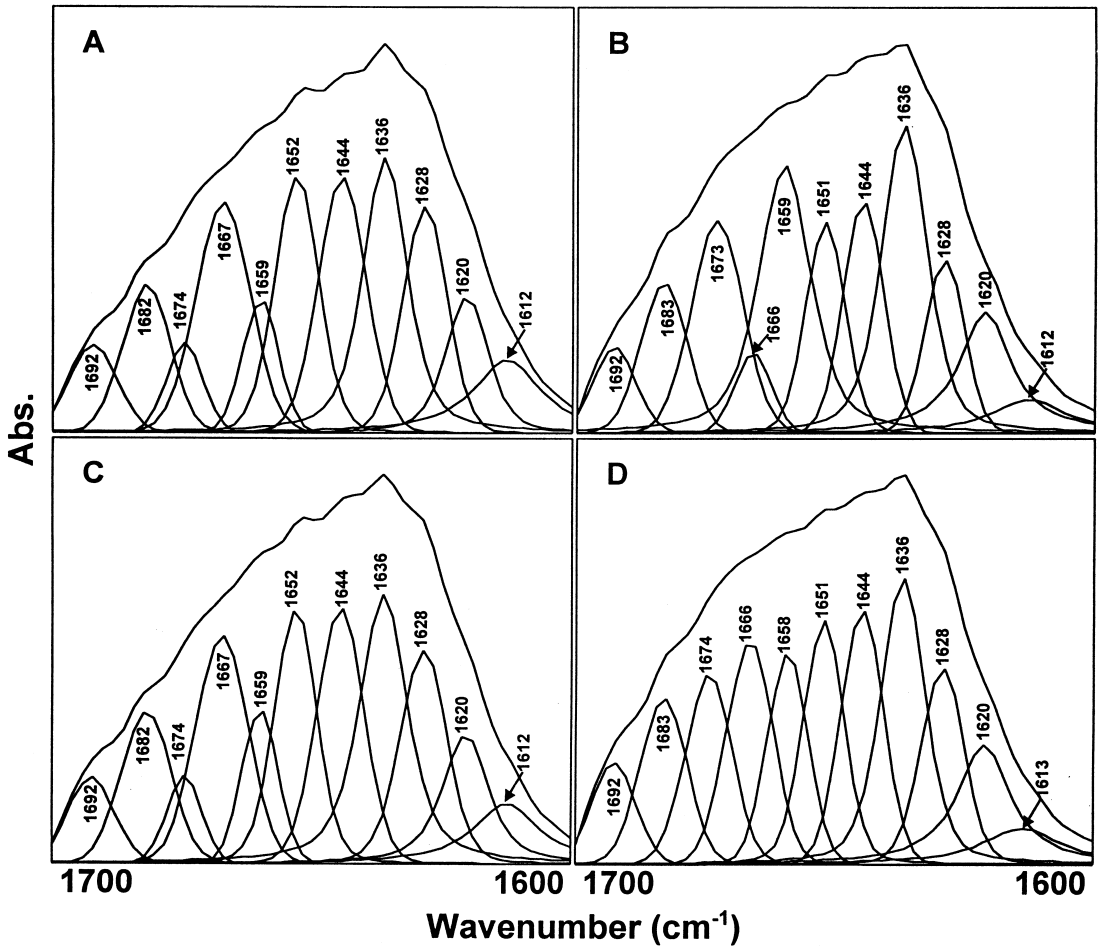

Fig. 5. Reflectance IR spectra and the best-fitting individual component bands in the 1700–1600 cm^{-1} region of the amide I band of the α-crystallin membrane. Key: A, native α-crystallin membrane without heating (25°C); B, native α-crystallin membrane after first-heating to 120°C; C, b sample after cooling to 25°C; D, c sample after reheating to 80°C.

ner two-layer structure in the three-layer model is highly stable, but the outer layer dissociates from the inner two layers at 35–45°C. Furthermore, at higher temperatures (62°C) the two-layer structure can rapidly reassociate to form a more compact conformation [27]. Interestingly, the present study showed that the first-heated α-crystallin membrane during the reheating process exhibited a thermal behavior similar to that of the three-layer model proposed. This perhaps might be attributed to the original conformation of the native α-crystallin membrane during first-heating from 25 to 120°C which can be slightly modified due to a change in the secondary structure (Table 1). When the first-heated α-crystallin membrane sample was cooled from 120 to 25°C, the modified conformation restored to the original structure and located at the outer layer, but with a slightly loose form. Reheating this first-heated sample may dissociate the loose structure to show a transitional change at 35–50°C. The inner two-layer structure of the α-crystallin membrane via rearrangement and/or the reassociation process then became more compact with continuous heating, and exhibited a thermal transition at 55–70°C. It is interesting to note that the transitional temper-

Table 1
The contents of protein secondary conformational components of the α-crystallin membrane before and after heating treatments

Treatment	Composition (%)			
	β-Turn	α-Helix	Random coil	β-Sheet
α-Crystallin membrane at 25°C	33.71	12.70	13.28	40.31
α-Crystallin membrane heating to 60°C	40.22	10.27	11.16	38.35
α-Crystallin membrane heating to 120°C	44.70	9.35	10.29	35.66
120°C heated α-crystallin after cooling to 25°C	33.75	12.20	13.07	40.97
120°C heated α-crystallin after reheating to 80°C	41.35	10.95	11.27	36.43

ature at 35–50°C for the reheated α-crystallin membrane was approximately similar to the temperature for the formation of the hydrophobic surface of α-crystallin in the liquid state, the reason will be studied in the future.

In conclusion, the reflectance FT-IR/DSC microscopic data evidence that the protein secondary conformation of α-crystallin in the membrane state had high thermal stability and reversibility via the heating process. The thermal transition of α-crystallin membrane was easily determined at 60°C by reflectance FT-IR/DSC microscopy. Moreover, the heated α-crystallin membrane during the reheating process also exhibited unique thermal behavior, similar to that of the thermal performance of the proposed three-layer model of α-crystallin with a transitional temperature at 35–50 and 55–70°C.

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