CHAPTER V

EFFECT OF α-CRYSTALLIN ON THE REFOLDING OF LYSOZYME AND RIBONUCLEASE A
5.1. INTRODUCTION

Studying the conformational states of partially unfolded or folded target proteins, which can be recognized by molecular chaperones, is an important aspect in understanding the molecular mechanism of chaperone function. A parallel study from our laboratory showed that α-crystallin binds to the molten-globule state of carbonic anhydrase (Rajaraman et al., 1996). Das et al. (1996) have shown that the conformational state of γ-crystallin bound to α-crystallin is compact in nature. Das and Surewicz (1995b) reported that alpha-crystallin recognizes intermediates only on the unfolding pathway but not on the refolding pathway of proteins. In order to understand the conformational aspects of target proteins which interact with α-crystallin, we have investigated the effect of α-crystallin on the refolding of denatured-disulfide intact and denatured-reduced hen egg white lysozyme and bovine pancreatic RNase A (Raman et al., 1997).

5.2. MATERIALS AND METHODS

Hen egg white lysozyme, RNase A, cytidine 3':5' cyclic monophosphate (cyclic CMP) and DL-cystine hydrochloride were purchased from Sigma Chemical Company, USA. 8-anilinonaphthalene-1-sulfonic acid (ANS) was obtained from Aldrich Chemical Company, USA. Dithiothreitol (DTT) was purchased from SISCO Research Laboratories, India. Guanidinium chloride (GdmCl) was purchased from Serva, Heidelberg. Lysozyme was further purified as described by Saxena and Wetlaufer (1970).
5.2.1. Preparation of denatured and denatured-reduced enzymes

Denatured enzymes were prepared by dissolving the enzymes (11.5mg lysozyme and 12mg RNase A) in 1ml of 50mM Tris acetate buffer (pH 8.1) containing 6.5M GdmCl. The sample was incubated at 25°C for about 16hrs. Denatured-reduced enzymes were prepared similarly with the buffer containing 80mM DTT.

5.2.2. Refolding experiments

Refolding of the denatured enzymes was performed by a 100-fold dilution of the denatured enzyme into 100mM Tris acetate buffer pH 8.1 (refolding buffer) either in the absence or in the presence of α-crystallin (0.5-1.0mg/ml) at 25°C or at 45°C. Small aliquots were withdrawn at different time intervals and the enzyme activity was measured. Refolding of denatured-reduced enzymes was performed similarly in the absence or in the presence of 0.5mg/ml α-crystallin, by a 100-fold dilution of the denatured-reduced stock into the refolding buffer that also contained 1 mM cystine hydrochloride at 37°C.

5.2.3. Circular dichroism and ANS binding experiments

Refolding of denatured-reduced RNase A was performed as mentioned above in the refolding buffer alone (without α-crystallin and cystine hydrochloride) and the far UV circular dichroism (CD) spectrum of this sample was recorded between 3 and 6 min after initiation of the refolding. CD spectra were recorded using a JASCO J-715 spectropolarimeter. CD spectra of native and denatured RNase A (in 6.0M GdmCl) were also recorded.
Fluorescence spectra of native of RNase A and lysozyme were recorded in the presence of 50µM ANS using a Hitachi F-4000 fluorescence spectrophotometer. The fluorescence of ANS bound to the refolding lysozyme or RNase A was measured by adding ANS 3min after the initiation of refolding of the denatured-reduced enzymes into the buffer lacking cystine hydrochloride. The excitation wavelength was set at 365nm. The excitation and emission band passes were set at 5nm. All spectra were recorded in correct spectrum mode.

5.2.4. Enzyme assay

Lysozyme activity was determined at 25 °C as described by Fischer et al. (1992a). The rate of enzymatic lysis of *M. lysodeikticus* cells, suspended in 0.1 M phosphate buffer (pH 6.3), was obtained by measuring the decrease in turbidity of the cell suspension at 450nm as a function of time using a Hitachi U-2000 UV-Visible spectrophotometer.

The activity of RNase A was measured using the method described by Crook et al. (1960) using cyclic CMP as substrate. 10µg of RNase A sample was added to 0.1mg/ml of cyclic CMP in 100mM Tris HCl buffer (pH 7.13) (the ionic strength adjusted to 0.2 with NaCl) and the increase in optical density at 284nm was monitored as a function of time. The rate of increase in the optical density is the measure of the activity of the enzyme. The percentage renaturation yields in the refolding studies of the enzymes were calculated with respect to the activity of the native enzymes.
5.3. RESULTS AND DISCUSSION

The formation of chaperone-target protein complex which prevents the aggregation of the partially folded states of the target proteins is an important first step in the chaperone function. In order to understand the conformational aspects of target proteins which interact with α-crystallin, we have investigated the effect of α-crystallin on the refolding process of denatured-disulfide intact and denatured-reduced hen egg white lysozyme and bovine pancreatic RNase A.

Lysozyme is one of the most extensively studied enzymes for its refolding properties (Saxena and Wetlauffer, 1970; Anderson and Wetlauffer, 1976; Perraudin et al., 1983; Goldberg et al., 1991; Fischer et al., 1992b; Fischer et al., 1993; Raman et al., 1996). As the concentration of the enzyme to be refolded increases the renaturation yield drastically decreases due to aggregation (Goldberg et al., 1991). Refolding of denatured lysozyme even at 1 mg/ml concentration does not result in any aggregation and almost 100% activity of the enzyme is recovered. However denatured-reduced lysozyme aggregates even at 50 μg/ml. We found that under appropriate concentrations of thiol/disulfide redox reagents, lysozyme can be refolded at high concentrations with high renaturation yields (Raman et al., 1996).

Table 5.1 shows the percentage recovery of activity of lysozyme upon refolding from its denatured state in the absence and in the presence of α-crystallin. The denatured-disulfide intact lysozyme refolds to its active state within a short time period (e.g., 1 min.). α-Crystallin does not have significant effect on the renaturation yield. Since α-crystallin exhibits temperature-induced structural alterations which are important in its chaperone-like activity
Table 5.1

Refolding of denatured lysozyme (at 0.115 mg/ml) and RNase A (at 0.12 mg/ml) in the absence and in the presence of 1 mg/ml α-crystallin.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Time (min.)</th>
<th>% Recovery of activity *</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>25°C -α</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>RNase A</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>83</td>
</tr>
</tbody>
</table>

*The % recovery of activity is with respect to the activity of the native enzymes.*
(Raman and Rao, 1994; Raman et al., 1995a; Raman and Rao, 1997), we have performed the experiment at 45°C. Even at this temperature, α-crystallin does not affect the renaturation yield.

Refolding of denatured-reduced lysozyme in the absence of disulfide exchange reagents at 0.15mg/ml by a 100-fold dilution into the refolding buffer alone, resulted in aggregation and yielded a turbidity value of 0.83 (measured as optical density at 500nm). On the other hand, refolding of denatured-reduced enzyme similarly, in the buffer that also contains 0.5mg/ml α-crystallin results in much reduced aggregation and yields a turbidity value of only 0.3. The resultant solution does not exhibit the enzyme activity. Thus, α-crystallin prevents the aggregation of the enzyme significantly upon refolding it from its denatured-reduced state.

We have earlier studied the refolding of denatured and denatured-reduced lysozyme (Raman et al., 1996). We found that refolding of denatured-reduced lysozyme (at 0.1mg/ml) in the absence of thiol-disulfide reagents leads to aggregation and loss of activity. This aggregation is negligible at the earlier time period (<10min.) after initiation of the refolding of the protein. Fluorescence and far UV-CD studies on this sample between 2-5min after initiation of the refolding have shown that the refolding pathway of denatured-reduced lysozyme involves an aggregation-prone intermediate state which possesses significant amount of secondary structure while lacking tertiary structure (as judged by fluorescence). Since the aggregation is high at high concentration of the enzyme and near UV-CD study requires high concentration of the enzyme, we performed refolding at high concentrations in the presence of low concentrations of urea in the refolding buffer (we found that urea prevents the aggregation of the intermediate). Lysozyme does not lose its native structure
even in 7M urea (Porubcan et al., 1978). The far and near UV-CD spectra of the intermediate in 2M urea are shown in figure 5.1. This intermediate binds the hydrophobic dye, ANS with greater avidity compared to the native or fully unfolded enzyme (Raman et al, 1996 and figure 5.4B) as usually observed for the molten-globule state of proteins (Semisotnov et al., 1991). Thus, these properties of the intermediate is similar to that of the molten-globule state of proteins. This aggregation prone intermediate can be oxidatively refolded to its active form in the presence of disulfide reagents such as cystine or GSSG (Raman et al., 1996). When the refolding buffer contains cystine, the aggregation is not observed due to the oxidative refolding of the intermediate. Hence, we have investigated the effect of α-crystallin under the condition which results in productive folding of the enzyme to its native state.

Figure 5.2 shows that the denatured-reduced lysozyme refolds with about 80% renaturation yield in the absence of α-crystallin in the presence of 1mM cystine hydrochloride as a disulfide exchange catalyst. The presence of α-crystallin inhibits this oxidative renaturation as shown in the figure. This suggests that α-crystallin interacts with the refolding-competent intermediate of lysozyme, preventing the aggregation of this intermediate as well as the oxidative refolding of denatured-reduced lysozyme. Such an inhibition of regeneration of activity of an enzyme by α-crystallin under conditions which favour renaturation of the enzyme has not been shown earlier. GroEL is known to inhibit the reactivation of chicken dihydrofolate reductase, which can otherwise refold to its active state, by forming a complex (Martin et al., 1991). The fact that α-crystallin does not inhibit the regeneration of lysozyme upon refolding from its denatured-disulfide intact state suggests that a molten-globule-like intermediate on its refolding pathway is either not present or not sufficiently long-lived to interact with α-crystallin.
Figure 5.1. Near (A) and far (B) UV-CD spectra of lysozyme. Lysozyme in 100mM Tris acetate buffer (pH 8.1) containing 2M urea; denatured-reduced enzyme in the buffer containing 2M urea; and denatured enzyme in the buffer containing 5.2 MGdmCl.
Figure 5.2: Oxidative refolding of denatured-reduced lysozyme (---) and RNase A (-------) at 0.115 mg/ml and 0.12mg/ml respectively in the absence (o) and in the presence (●) of 0.5mg/ml α-crystallin at 37°C. The refolding buffer, 100mM Tris-acetate (pH 8.1) also contained 1mM cystine hydrochloride. The percentage recovery of activity is with respect to the activity of the native enzyme.
We have also studied the effect of α-crystallin on the refolding of RNase A, an enzyme which has been shown to refold to its native state (Anfinsen, 1973). It is evident from table 5.1 and figure 5.2 that α-crystallin does not inhibit the reactivation of RNase A upon refolding the enzyme either from its denatured-disulfide intact or denatured-reduced states. In order to understand the molecular basis of the differential behaviour of α-crystallin towards the oxidative refolding of lysozyme and RNase A, we have investigated circular dichroism and ANS binding of a sample of denatured-reduced RNase A between 3 and 6 min of initiation of refolding by diluting into the refolding buffer containing no cystine. Figure 5.3 shows the far UV-CD spectrum of this sample along with the spectra of the native and denatured enzyme (in 6.0M GdmCl). Unlike in the case of lysozyme, denatured-reduced RNase A within 3-6 min of its refolding shows an extended conformation. Figure 5.4A shows that this state of RNase A binds the hydrophobic dye ANS only marginally higher compared to its native state. On the other hand, the intermediate state of lysozyme exhibits 7-8-fold increased ANS binding compared to its native state (Figure 5.4B). These results suggest that α-crystallin does not interact with the state of RNase A having a more extended conformation (randomly coiled) and less solvent exposed hydrophobic surfaces. These results also suggest the absence of a compact, hydrophobic intermediate on the refolding pathway of RNase A which could interact with α-crystallin. This explains the lack of any effect of α-crystallin on the refolding of RNase A. Thus, it appears that α-crystallin does not interact with extended conformational states of target proteins. This conclusion is in agreement with the observation that α-crystallin does not form a stable complex with reduced carboxymethylated α-lactalbumin which assumes an extended conformation (Carver et al., 1995).
Figure 5.3: Far UV-CD spectra of native (-----), denatured or denatured-reduced states (in 6.0M GdmCl) (.........) of RNase A and the refolding RNase A (-------) (obtained between 3 and 6min of initiation of refolding of denatured-reduced enzyme by 100-fold dilution into the refolding buffer lacking cystine hydrochloride).
Figure 5.4: Fluorescence spectra of ANS bound to RNase A (A) and lysozyme (B). Native (-----) and the refolding enzymes (--------) (obtained between 3 and 6 min of initiation of refolding of denatured-reduced enzymes by 100-fold dilution into the refolding buffer lacking cystine hydrochloride). 50μM ANS was added 3min after initiation of refolding and spectra recorded within 6min of the initiation of the refolding. The fluorescence intensity is normalised with respect to the fluorescence of the respective native enzymes.
Das and Surewicz (1995b) studied the chaperone-like activity of α-crystallin towards the thermal- and refolding-induced aggregation of rhodanese and found that it prevents the thermal-aggregation of rhodanese at 47°C but does not prevent the refolding-induced aggregation of the protein at 25°C. They concluded that, unlike other molecular chaperones, α-crystallin recognizes non-native intermediates of proteins only on their denaturing (unfolding) pathway but not on their refolding pathway. Our results on the refolding of denatured-reduced lysozyme, on the other hand, show that α-crystallin does recognize the refolding intermediates. Our studies (chapter II & III) show that the chaperone-like activity of α-crystallin is enhanced several fold above 30°C and that subtle changes in tertiary and/or quaternary structure of α-crystallin which lead to enhanced exposure of its hydrophobic surfaces are important in its chaperone-like activity. Thus, the apparent discrepancy between our results and those of Das and Surewicz (1995b) can be explained on the basis of the temperature at which the experiments were conducted. We have also observed that the aggregation of the βL-crystallin upon its refolding is not prevented significantly at temperatures below 30°C and is prevented above this temperature (chapter I) suggesting that α-crystallin interacts with refolding intermediates of βL-crystallin and prevents their aggregation. It appears that some specificity is involved in the interaction of chaperone and substrate proteins. GroEL is known to bind to flexible molten-globule state of rhodanese and chicken dihydrofolate reductase (Martin et al., 1991) but not to interact strongly with α-lactalbumin either in its more-compact molten-globule state or in its reduced state which assumes an extended conformation (Okazaki et al., 1994). The chaperones from Hsp70 family bind to extended conformations (Landry et al., 1992). Our studies show that α-crystallin inhibits the aggregation as well as the oxidative renaturation of lysozyme by binding to the refolding intermediate which has substantial amount of secondary
structure with no tertiary structure but with more solvent-exposed hydrophobic surfaces compared to the native enzyme. On the other hand, it does not inhibit the refolding of RNase A: α-Crystallin does not interact strongly with the refolding enzyme which is in an extended conformation with negligible solvent exposed hydrophobic surfaces.

5.4. CONCLUSIONS

(i) α-Crystallin inhibits the oxidative reactivation of denatured-reduced lysozyme, but has no effect on the reactivation of denatured or denatured-reduced RNase A.

(iii) The refolding intermediate of denatured-reduced lysozyme exhibits significant amount of secondary structure, highly solvent exposed hydrophobic surfaces with no rigid tertiary structure. On the other hand, denatured-reduced RNase A, on its refolding, exhibits extended conformation with only marginally higher solvent exposed hydrophobic surface compared to the native state.

(iv) Our results suggest that α-crystallin does not interact strongly with the extended conformation of the target protein but interacts with unfolded state(s) of the target protein possessing significant secondary structure with highly solvent exposed hydrophobic surfaces.
CONCLUDING REMARKS
α-Crystallin prevents the aggregation of other crystallins and of several enzymes like a molecular chaperone (Horwitz, 1992). Consequently, several groups including our own have been investigating the molecular mechanism of the chaperone-like property of α-crystallin and its relevance to eye lens transparency and cataract. Most of the other workers studied the chaperone-like activity of α-crystallin using the aggregation model system where the aggregation of the target protein is induced by temperature (usually at elevated temperatures). We have studied the chaperone-like property of α-crystallin using model systems where the aggregation of target proteins is achieved by non-thermal modes. In general, studies on the chaperone-like activity of α-crystallin from our laboratory concern with two aspects; (i) the structural changes of α-crystallin which are important in its chaperone-like activity and (ii) the conformational states of target proteins which are recognised by α-crystallin.

UV light has been shown to be one of the major risk factors in the development of cataract. Among the mammalian lens crystallins, γ-crystallin is vulnerable to UV radiation; it aggregates and precipitates upon irradiation with UV-light (Chakrabarti, et al., 1986; Mandal et al., 1988; Walker and Borkman, 1989). We have, therefore, investigated the chaperone-like activity of α-crystallin towards the photo-aggregation of γ-crystallin (Rao et al., 1993; Raman and Rao, 1994). At physiological temperature, α-crystallin prevents the photo-aggregation of γ-crystallin significantly, suggesting a chaperone-like role of α-crystallin in the lens. However, the protective ability of α-crystallin is found to be dependent on temperature: it prevents the photo-aggregation of γ-crystallin less significantly at temperatures below 30°C and more significantly above this temperature. The major force in the interaction between the target protein and α-crystallin is found to be hydrophobic in
nature. Based on these studies and preliminary studies on the structural changes of \( \alpha \)-
crystallin, we have hypothesised that \( \alpha \)-crystallin prevents the aggregation of non-native
structures of other proteins by providing appropriately placed hydrophobic surfaces. A
structural transition above 30\(^\circ\)C enhances the protective ability of \( \alpha \)-crystallin, perhaps by
increasing or reorganizing the hydrophobic surfaces (Raman and Rao, 1994).

Our studies on the chaperone-like activity of \( \alpha \)-crystallin towards the aggregation of
insulin and zeta-crystallin (Raman et al., 1995a) and on the rapid refolding of \( \alpha \)-, \( \beta \)- and \( \gamma \)-
crystallins (Raman et al., 1995b; Raman and Rao, 1997) support the above hypothesis.
Subsequent studies from other laboratories (Das and Surewicz, 1995; Smith et al., 1996) also
support our hypothesis. These studies led us to investigate in detail the structural changes of
\( \alpha \)-crystallin in the context of its chaperone-like function (Raman and Rao, 1997) which was
poorly understood earlier. Temperature is known to modulate the chaperone function of
other molecular chaperones such as GroEL, HSP90 and DnaK or HSP70 (Brunschiwer et al.,
1993; Hansen and Gafni, 1994; Hayer Hartl et al., 1996; Yonehara et al., 1996).

\( \alpha \)-Crystallin has been shown to chaperone the assembly of intermediate filaments
(Nicholl and Quinlan, 1994). It also stabilizes actin filaments and prevents cytochalasin-
induced depolymerization in a phosphorylation-dependent manner (Wang and Spector, 1996).
\( \alpha \)-Crystallin has been found to inhibit the fructation-induced inactivation of glutathione
reductase (Blakytny and Harding, 1996), carbamylation-induced inactivation of 6-
phosphogluconate dehydrogenase (Ganea and Harding, 1996), glycation-induced inactivation
of malate dehydrogenase (Heath et al., 1996) and steroid-induced inactivation of catalase
(Hook and Harding, 1996). However, the mechanism of such inhibition is not clear.
Equilibrium binding studies show that α-crystallin binds ATP (Palmisano et al., 1995) but does not appear to have ATPase activity. Several post-translational modifications such as glycation, deamidation, cross linking etc., appear to affect the chaperone-like activity of α-crystallin (Cherian and Abraham, 1995; van Boekel et al., 1995; Sharma and Ortwerth, 1995). However, phosphorylation of α-crystallin does not appear to have any effect on its activity (Wang et al., 1995). Photolysis of α-crystallin with UV-light decreases its chaperone-like activity (Borkman and McLaughlin, 1995; Ellozy et al., 1996). Immobilization of the C-terminal extension of bovine αA-crystallin reduces chaperone-like activity suggesting the involvement of the C-terminal extension in its activity (Smulder et al., 1996). Site directed mutagenesis studies show that mutation of Asp69 to Ser affects the chaperone-like activity of αA-crystallin. α-Crystallin from old human lenses (Cherian and Abraham, 1995) and from selenite-induced cataractous lenses of an animal model (Kelley et al., 1993) are found to exhibit decreased chaperone-like activity. Our study on refolding of crystallins at high concentrations (Raman et al., 1995b) shows that β- and γ- crystallins aggregate upon refolding while α-crystallin does not aggregate. Co-refolding of β-crystallin or γ-crystallin with α-crystallin prevents the aggregation under similar conditions. All these studies provide evidences that the chaperone-like activity of α-crystallin is important in the formation and maintenance of the transparency of the eye lens. In fact, a recent study of Brady et al. (1997) shows that targeted disruption of the mouse αA-crystallin gene induces cataract. Mutations or environmental changes that destabilise the native states of proteins or divert them from their normal folding pathway lead to aggregation of proteins in vivo and disease (see the table, Wetzel, 1994; Thomas et al., 1995; Sifers, 1995). Cataract is now considered as a "Chaperone-disease" - the failure of the chaperone, α-crystallin, in preventing the aggregation of other crystallins due to their age-related conformational changes manifests in
<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutant protein/protein involved</th>
<th>Molecular phenotype</th>
</tr>
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<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>CFTR</td>
<td>Misfolding/altered Hsp70 and calnexin interactions</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td>Fibrillin</td>
<td>Misfolding</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Superoxide dismutase</td>
<td>Misfolding</td>
</tr>
<tr>
<td>Scurvy</td>
<td>Collagen</td>
<td>Misfolding</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>α-Ketoacid dehydrogenase complex</td>
<td>Misassembly/misfolding</td>
</tr>
<tr>
<td>Cancer</td>
<td>p53</td>
<td>Misfolding/altered Hsp70 interaction</td>
</tr>
<tr>
<td>Osteogenesis imperfecta</td>
<td>Type I procollagen pro α</td>
<td>Misassembly/altered BiP expression</td>
</tr>
<tr>
<td>Scrapie/Creutzfeldt-Jakob/familial insomnia</td>
<td>Prion protein</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>β-Amyloid</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Familial amyloidosis</td>
<td>Transthyretin/lysozyme</td>
<td>Aggregation</td>
</tr>
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<td>Cataracts</td>
<td>Crystallins</td>
<td>Aggregation</td>
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<td>Familial hypercholesterolemia</td>
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<td>Improper trafficking (mislocation)</td>
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Understanding the conformational states of partially unfolded or folded target proteins which can be recognized by molecular chaperones is one of the important aspects in understanding the molecular mechanism of chaperone function. A few studies have attempted to find out the conformational states of target proteins which bind to \( \alpha \)-crystallin. An earlier study from our laboratory showed that \( \alpha \)-crystallin binds to the molten-globule state of carbonic anhydrase (Rajaraman et al., 1996). Das et al. (1996) have shown that the conformational state of \( \gamma \)-crystallin bound to \( \alpha \)-crystallin is compact in nature. Das and Surewicz (1995b) reported that \( \alpha \)-crystallin only recognizes intermediates on the unfolding pathway but not on the refolding pathway of proteins. We have investigated the refolding of hen egg white lysozyme and RNase A and the effect of \( \alpha \)-crystallin on the refolding of these enzymes. We have found that an aggregation prone intermediate on the refolding pathway of lysozyme which possesses significant amount of secondary structure but lacks tertiary structure, interacts with \( \alpha \)-crystallin; on the other hand the extended conformation of RNase A does not interact with \( \alpha \)-crystallin. We have also found that the aggregation of the \( \beta_L \)-crystallin upon its refolding is not prevented significantly at temperatures below 30°C and is prevented above this temperature (Raman and Rao, 1997) suggesting that \( \alpha \)-crystallin interacts with refolding intermediates of \( \beta_L \)-crystallin and prevents its aggregation. Thus, our studies show that \( \alpha \)-crystallin interacts with refolding intermediates as well.

It was earlier believed that molecular chaperones recognize, in general, all non-native structures of substrate proteins. However, it is now becoming clear that some specificity is involved in the interaction of chaperone and substrate proteins. GroEL is
known to bind to the flexible molten-globule state of rhodanese and chicken dihydrofolate reductase (Martin et al., 1991) but not to interact strongly with α-lactalbumin either in its more-compact molten-globule state or in its reduced state which assumes an extended conformation (Okazaki et al., 1994). The chaperones from the Hsp70 family bind to extended conformations (Landry et al., 1992). Our studies show that α-crystallin inhibits the aggregation as well as the oxidative renaturation of lysozyme by binding to the refolding intermediate of denatured-reduced lysozyme which has substantial amount of secondary structure, no tertiary structure, and has more solvent-exposed hydrophobic surfaces compared to the native enzyme. On the other hand, it does not inhibit the refolding of RNase A. This may be because α-crystallin does not interact strongly with the refolding enzyme which is in an extended conformation with negligible solvent exposed hydrophobic surfaces. These studies are useful in understanding of chaperone-substrate interactions.