The eye lens is a transparent, highly refractive tissue that focuses light to form images on the retina. The lens originates from ectodermal cells; posterior epithelial cells elongate and reach the anterior cell layer to form fibre cells which constitute main body of the lens. The anterior epithelial cells remain as such. The fibre cells are permanent and the newly grown cells are layered on the previously existing ones so that the older cells are always found towards the core (nucleus) and the younger ones towards the periphery (cortex) of the lens. During lens development, a class of proteins called crystallins are synthesized and reach high (0.2-0.5 g/ml) cytoplasmic concentrations. The high protein concentration provides the required refractive index, while variation in concentration and distribution of the crystallin species leads to a gradual increase in refractive index from the periphery to the centre. The lens transparency is thought to originate from the proper organization of the fibre cells and the intercrystallin interactions. Since membranes of lens fibre cells contain membrane proteins and other proteins associated with them, their average refractive index might well be close to that of the surrounding crystallin rich cytoplasm; this would result in only a low level of membrane scattering (Tardieu, 1988). X-ray scattering measurements reveal the existence of short range ordered liquid organisation of the crystallins in the cytoplasm (Tardieu, 1988). Thus inter-crystallin interactions appear to be important for the lens transparency.

1.1. Cataract and factors associated with it

Factors that affect lens transparency and create fluctuations in the lens refractive index lead to visual impairment, the condition called cataract. "Transparent" qualifies a medium that allows light to pass through. Extinction or removal of light arises from two physical phenomena, absorption and scattering, both of which reduce the amount of light that
is transmitted past a medium. Therefore, factors that increase either the absorption or the scattering in the eye lens would lead to loss of transparency. Due to low metabolism and lack of turnover, the damages or modifications that occur to the lens constituents accumulate with age.

1.1.1. Increased absorption of light in the lens

Epidemiological studies identify various risk factors, apart from ageing, like nutrition, diabetes mellitus, trace metals, ultraviolet radiation, smoking etc., in the causation of cataract (Mohan et al., 1989; Balasubramanian et al., 1993a). Tropical regions which have high incidence of sunlight (also higher UV radiation) have been shown to have higher prevalence and early onset of age-related cataract (Zigman et al., 1979; Hollows and Moran, 1981). Many types of cataract are associated with brunescence. Pigmentation of the lens (Zigman, 1971) not only contributes to the absorption of light by the lens but also leads to further damage of the lens contents. Pigments in the lens are believed to be generated through oxidative damage.

Photo-oxidative damage occurs by either direct photolysis or photosensitization. Direct photolysis (by UV light) of aromatic amino acids, particularly tryptophan residues, of the lens proteins leads to the generation of various photoproducts such as N-formylkynurenine (NFK), kynurenine (Ky), 3-hydroxykynurenine (3HK) and the glucoside of 3HK. These compounds impart colour to the lens; diffuse reflectance spectroscopic studies show that pale yellow cataractous lenses exhibit an absorption band at 365nm similar to that of 3HK and that brunescent cataracts with high level of pigmentation show an absorption peak over 400nm, possibly due to a dimerized product of 3HK, called xanthommatin.
A recent study shows the presence of quinoline derivatives in brunescent lenses (Luthra et al., 1994). Many of these compounds act as photosensitizers. In the presence of oxygen they are capable of generating active species of oxygen such as singlet oxygen (\( ^1\text{O}_2 \)), superoxide (\( \text{O}^2^- \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and the hydroxyl radical (.OH) (Grossweiner, 1984; Creed, 1984a&b; Walrant et al., 1975; Balasubramanian et al., 1990). In photo-sensitization phenomenon, the sensitizer molecule is promoted to its excited singlet state by the absorption of light; the process of intersystem crossing then takes the system to a longer lived triplet state. From the triplet state of the sensitizer there are two major mechanisms which can lead to oxidation of substrate molecules. The type I or direct reaction pathway is a redox reaction of the triplet sensitizer involving either electron transfer or proton abstraction from the substrate by the sensitizer, with the production of active oxygen species such as \( \text{O}^2^- \), \( \text{H}_2\text{O}_2 \) and .OH. These radicals can rapidly react with the substrate (membrane lipids, proteins etc.) and generate modifications including intermolecular crosslinking (Andley and Clark, 1989a,b; Varma and Mooney, 1986). In the type II reaction, the triplet sensitizer transfers its excited state energy not to the substrate but to ground state molecular oxygen, producing a highly reactive singlet oxygen (\( ^1\text{O}_2 \)), which can then react with or oxidize the substrate (Goosey et al., 1980; Zigler and Goosey, 1984). Ascorbate and metal ions such as \( \text{Cu}^{2+} \) and \( \text{Fe}^{3+} \) in aerobic conditions have been shown to generate radicals that can oxidize lens constituents (Garland et al., 1988). In the presence of transition metal ions, ascorbate is oxidized to dehydroascorbate generating active oxygen species via the Fenton reaction (McCord and Day, 1978; Halliwell, 1978).

In most of the cataracts associated with diabetic hyperglycemia, colouration of the lens is observed. Sugars undergo autooxidation to form dicarbonyl compounds and hydrogen peroxide via reactive intermediates of dioxygen and carbon-centered free radicals (Thornalley
et al., 1984a,b). Increased non-enzymatic glycation can be seen in crystallins incubated in various sugars along with the formation of products of non-tryptophan fluorescence (Liang and Rossi, 1990). Studies on the mechanisms of glycation indicate the participation of lysine residues which can form Schiff-base stabilized by Amadori rearrangement. An yellow advanced glycosylation end product (Amadori product) has been isolated (Pongor et al., 1984; Njorge et al., 1989). Degradation of Amadori product and subsequent reactions lead to the production of brown fluorescent pigments called advanced Maillard products and crosslinks which are thought to accumulate in long-lived crystallins (Monnier and Cerami, 1981). Several glycated products that were isolated include N-α-formyl-ɛ-fructoselysine, N-ɛ-carboxymethyllysine and hexitol lysine (Ahmed et al., 1986).

Smoke is one of the risk factors associated with cataract (Mohan et al., 1989). Smoke contains water soluble polycyclic aromatic hydrocarbons which can cause oxidative stress on the lens by generating reactive oxygen species (Balasubramanian et al., 1993b; Shalini et al., 1994). Smoke condensate is shown to affect membrane transport, particularly the K+ pump which leads to opacification of lenses in organ culture (Rao et al., 1995).

1.1.2. Increased light scattering in the lens

Protein aggregation with or without phase separation is the major cause of increased light scattering in the lens. Post-translational modifications of crystallins involving deamidation, carbamylation, non-enzymatic glycosylation and oxidation lead to a decreased solubility of the crystallins as a function of age (Hoenders and Bloemendal, 1981) accompanied by protein cross-linking. These modifications to the crystallins affect their conformation (and stability) and lead to packing alterations. Lenses exposed to UV-radiation
showed much reduced fragmental mobility compared to normal lenses (Rao et al., 1989). Such packing alterations and the aggregation would lead to the formation of scattering centers. In addition, electrolytic imbalance is also shown to play a role in the increased light scattering. Low sodium and calcium concentrations and a high potassium concentration (Duncan and Jacob, 1984) are maintained through active transport mechanisms and gap junctions between the fibre cells (Benedetti et al., 1981). Alterations in transport mechanism and interfibre cell communications lead to lens opacification. Elevated lens cytosolic calcium concentration has been correlated with cataract formation in both human and animal models (Iwata, 1974; Lohmann et al., 1986; Shearer et al., 1987). Apart from glycation of crystallins and the formation of pigments in cataracts due to hyperglycimia, fluid filled vacuoles are also observed which scatter light (Kuriyama et al., 1983). The glucose present in the lens is converted by the enzyme, aldose reductase, into sorbitol. The polyols accumulate in the cells and prompt swelling and disruption of normal cellular architecture.

1.2. Crystallins

Crystallins contribute about 35% of the wet weight and about 90% of the dry weight of the lens. Crystallins are classified as α, β, γ, δ etc., according to their amino acid sequence, charge and immunological properties. Mammalian lens contains α, β and γ crystallins whereas γ-crystallin is replaced by δ-crystallin in reptilian and avian lenses. Among these crystallins β- and δ- crystallins binds calcium ion and probably play an important role in the maintenance of intralenticular calcium level (Sharma et al., 1989). Protein sequence comparisons have shown that a number of taxon-specific crystallins are actually enzymes (see table 1.1) that have been probably recruited to function as structural proteins in the lens.
Table 1.1

Some properties of crystallins found in vertebrate eye lenses.

<table>
<thead>
<tr>
<th>Crystallin</th>
<th>Sub types</th>
<th>Occurrence in vertebrates</th>
<th>Molecular form</th>
<th>Sub unit Mol. Mass. (kDa)</th>
<th>pI</th>
<th>Homology to non-crystallin proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>2</td>
<td>all</td>
<td>40-50mer</td>
<td>20</td>
<td>4.8-5.0</td>
<td>Small HSPs</td>
</tr>
<tr>
<td>( \beta )</td>
<td>7</td>
<td>all</td>
<td>2-8 mer</td>
<td>23-35</td>
<td>5.7-7.1</td>
<td>Protein S of <em>Myxococcus xanthus</em></td>
</tr>
<tr>
<td>( \gamma )</td>
<td>6-7</td>
<td>except in birds and reptiles</td>
<td>monomer</td>
<td>20</td>
<td>7.1-8.1</td>
<td>Protein S of <em>Myxococcus xanthus</em></td>
</tr>
<tr>
<td>( \delta )</td>
<td>2</td>
<td>in all birds and reptiles</td>
<td>tetramer</td>
<td>48-50</td>
<td>4.9-5.3</td>
<td>Arginosuccinate lyase</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>1</td>
<td>some birds and reptiles</td>
<td>trimer</td>
<td>38</td>
<td>7.5</td>
<td>Lactate dehydrogenase B4</td>
</tr>
<tr>
<td>( \rho )</td>
<td>1</td>
<td>in frogs</td>
<td>monomer</td>
<td>33-35</td>
<td>8.5</td>
<td>Aldehyde and aldose reductase; Prostaglandin F synthase</td>
</tr>
<tr>
<td>( \tau )</td>
<td>1</td>
<td>some fishes, birds and reptiles</td>
<td>monomer</td>
<td>46-48</td>
<td>7.0-8.0</td>
<td>( \alpha )-Enolase</td>
</tr>
<tr>
<td>( \lambda )</td>
<td></td>
<td>rabbits and hares</td>
<td></td>
<td>35</td>
<td></td>
<td>Hydroxy acyl CoA dehydrogenase</td>
</tr>
<tr>
<td>( \zeta )</td>
<td>1</td>
<td>guinea pigs</td>
<td>monomer</td>
<td>36</td>
<td></td>
<td>Alcohol dehydrogenase</td>
</tr>
</tbody>
</table>
(Piatigorsky and Wistow, 1989). Whether these enzyme crystallins function as enzymes in the lens, however, is not determined. The properties of various crystallins present in different species are listed in table 1.1.

\[ \gamma \text{-Crystallin exists as monomers of molecular mass ranging from 20-27 kDa. It can be resolved into five components with isoelectric points greater than 7.0. One of its isoforms called } \gamma II \text{ crystallin is a cryoprotein: it precipitates when the temperature is lowered below 10°C due to phase separation and goes back into solution when the temperature is raised. } \beta \text{-Crystallin exists as a heterogeneous multimeric protein with subunit molecular masses ranging from 23-35 kDa. } \beta \text{-Crystallins comprise a multigene family of basic } [\beta B1, \beta B2, \beta B3] \text{ and acidic } [\beta A1, \beta A2, \beta A3, \beta A4] \text{ polypeptides (Berbers et al., 1984). } \beta B2\text{-Crystallin previously known as } \beta Bp, \text{ is the predominant subunit in hetero oligomers; it is versatile in its interactions, being able to self-associate into dimers or interact with other acidic and basic subunits to form dimer and larger multimers (Slingby and Bateman, 1990). These polypeptide chains occur as multimers, which can be resolved by gel filtration into two size classes, } \beta H \text{ (high) and } \beta L \text{ (low) (Harding and Dilley, 1976; Bloemendal, 1977). } \beta L \text{ contains dimers and trimers while } \beta H \text{ has an oligomeric structure, probably consisting of up to 7 or 8 subunits (Bindels et al., 1981). X-ray crystal structures are available for } \beta B2 \text{ and } \gamma II \text{ crystallins (Bax et al., 1990; Blundell et al., 1981). The } \beta \text{- and } \gamma \text{-crystallins are structurally similar to each other in having two domains linked by a connecting peptide that is extended in the } \beta \text{-crystallins, but bent in } \gamma \text{-crystallin (Bax et al., 1990). This arrangement leads to a different orientation of the two domains in each class such that they are in contact in } \gamma \text{-crystallin but not in the } \beta \text{-crystallins. The oligomeric bovine } \beta \text{-crystallin sequences have between 45% and 60% identity with each other for regions corresponding to the globular domains and about} \]
30% identical residues with the monomeric γ-crystallin (Bax et al., 1990). An important feature of β-crystallin structure that emerges from the sequences is the variability of the N-terminal extension. The sequence data from the different members of the β-crystallin family showed that if N and C termini are excluded, then the level of homology among basic β-crystallin members is around 60% as compared with ~ 50% if a basic member is compared with acidic member (Slingsby et al., 1988).

1.3. α-Crystallin

α-Crystallin is one of the most abundant of the crystallins, representing up to 35% of the total protein. It is also the largest, existing as a multimeric protein with an average molecular mass of 800-900 kDa (Bloemendal, 1981). Since α-crystallin is composed of two distinct gene products of αA and αB, each of ~ 20kDa in mass, the average aggregate of α-crystallin has around 40-45 subunits. The two types of subunits, αA and αB, crystallins comprise 173 and 175 amino acid residues respectively and show about 57% sequence homology with each other (Groenen et al. 1994). Bovine eye lens α-crystallin is made up of both types of subunits with the ratio of 3:1 (Siezen et al. 1978). The ratio varies with species (de Jong, 1981) and also with developmental age (Thomson and Augusteyn, 1985).

1.3.1. Presence of α-crystallin in non-lenticular tissues

Until about 1989, α-crystallin was believed to be a lens-specific, structural protein. Later, it was shown to exist in non-lenticular tissues such as kidney, brain, muscles, spleen etc. (Bhat and Nagineni, 1989; Dubin et al., 1989; Iwaki et al., 1989; Kato et al., 1991 a&b).
It appears that highest levels of αB-crystallin are observed in non-lenticular tissues with high levels of oxidative mitochondrial enzyme activity (Iwaki et al., 1993). Up to 2% of αB-crystallin is found in rat soleus muscle (Kato, et al., 1991a). The highest non-lenticular concentration of αA-crystallin (17ng/mg) has been detected in rat spleen (Kato, et al., 1991b). Expression of αB-crystallin can be induced by thermal (Klemenz et al., 1991) or hypertonic stress (Dasgupta et al., 1992). It is also expressed under various diseased conditions. It is expressed during development of benign tumors associated with tuberous sclerosis and the development of astrocytic tumors (Iwaki et al., 1991). Its expression may indeed be a useful biochemical marker for studying the pathogenesis of various types of human brain tumors (Aoyama et al., 1993). αB-crystallin is expressed at elevated levels in the brains of patients with Alzheimer's disease (Renkawek et al., 1994). The exact non-lenticular role of α-crystallin in normal and diseased tissues, however, is not understood.

1.3.2. Homology between α-crystallin and heat shock proteins

α-Crystallin subunits are homologous with the ubiquitous small heat shock proteins (HSPs)(Ingolia and Craig, 1982; de Jong, et al., 1988). The sequence similarity between HSPs and α-crystallin is more pronounced in the C-terminal parts of the polypeptides (Wistow, 1985). Sequence similarities have also been noticed between this domain and regions of the HSP70 (Lee et al., 1993). Klemenz et al., (1991) have showed that αB-crystallin in NIH 3T3 cells behaves in several ways like the small HSPs. Both small HSPs and α-crystallin form large aggregates. Mixed aggregates of αB-crystallin and small HSPs have been observed in vivo (Zantema et al., 1992; Kato et al., 1992). The secondary structure of calf α-crystallin and mouse HSP25 are very similar, both proteins having primarily β-sheet conformation (Merck
et al., 1993). As mentioned earlier, α-crystallin is also stress inducible like HSPs. Another common feature of α-crystallin and small HSPs is their phosphorylation on specific serine residues. Phosphorylation of α-crystallin in lens epithelium is reversible (Chiesa and Spector 1989) and is thought to be catalyzed by a cAMP-dependent kinase (Voorter et al., 1986; Chiesa et al., 1987). Phosphorylation of small HSPs occurs in response to a number of mitogenic and environmental stimuli (Arrigo and Welch, 1987; Saklatvala et al., 1991; Landry et al., 1991). α-Crystallin can also autophosphorylate serine in a cAMP-independent manner (Kantorow and Piatigorsky, 1994). However, the functional significance of phosphorylation of α-crystallin is not understood.

1.3.3. Structure of α-crystallin

Electron micrographs of α-crystallin reveal spherical aggregates with average diameters of around 140-150 Å (Siezen et al., 1980). The volume of this aggregate is calculated to be around $1.44 \times 10^6$ Å$^3$ (Carver et al., 1994). Circular dichroism studies showed that α-crystallin mainly contains β-pleated sheets and almost no α-helices (Li and Spector, 1974; Liang and Chakrabarti, 1982; Siezen and Argos, 1983). Fourier transformed infrared analyses showed the presence of 40-50% β-sheet and 5-10% α-helical structure (Lamba et al., 1993). The tertiary structure of α-crystallin is not known. Several hypothetical models have been suggested. On the basis of hydrophobic residue alignment of α-crystallin and β- and γ-crystallins, α-crystallin has been proposed to consist of six folding units, arranged in a two or three domain structure (Siezen, 1981). As for β- and γ-crystallins, a two domain structure has also been proposed for α-crystallin (Wistow, 1985; van den Oetelaar and Hoenders, 1989; Merck et al., 1992; Carver et al., 1993). Wistow (1985)
proposed the overall structure of α-crystallin to consist of a globular N-terminal domain of two symmetry related motifs and a somewhat larger C-terminal domain, also of two motifs with an exposed C-terminal arm, based on the gene structure and internal similarity of αA-crystallin. The N-terminal domain corresponds to exon 1 and the entire C-terminal region is encoded by exons 2 and 3. There is some experimental support based on unfolding studies for this model (van den Oetelaar and Hoenders, 1989; Merck et al., 1992; Carver et al., 1993). The quaternary structure of α-crystallin seems to be even more complicated. Several models have been proposed for the quaternary structure of α-crystallin - a three-layered structure (Tardieu et al., 1986; Siezen et al., 1980), a micelle-like structure (Augusteyn and Koretz, 1987), a combination of the micellar and three-layer model (Walsh et al., 1991), a rhombic dodecahedron (Wistow, 1993) and a pore like structure (Carver et al., 1994). However, none of these models explains all the properties of α-crystallin. In the absence of X-ray crystallographic data, the structure of α-crystallin and its packing remains speculative and is a matter of controversy.

1.3.4. Structural stability of α-crystallin

Among mammalian lens crystallins, α-crystallin is highly thermo stable. This unique thermal stability is not shown by β- and γ- crystallins, both of which precipitate at elevated temperatures. Maiti et al. (1988) reported that α-crystallin does not denature even at temperature as high as 100°C. Surewicz and Olesen (1995) have studied infrared spectra of α-crystallin and observed that it undergoes a major thermotropic transition at 60-62°C. This transition corresponds to the transition observed by Walsh et al. (1991) and our own
observation (Raman and Rao, 1994 & 1997). However, α-crystallin is not stable towards chemical denaturants such as urea and GdmCl. Also, its quaternary structure (in terms of molecular mass) is known to be altered under various experimental conditions. There have been several studies focused to understand the quaternary structure of α-crystallin and its alterations with temperature, metal ions, pH etc. (Tardieu et al., 1986; Siezen et al., 1980).

As mentioned earlier, α-crystallin is isolated as spherical molecules with diameter of about 15 nm and molecular weight of 800-900 kDa (S_{20,w} ~17-18S, also called as α_c-crystallin) (Siezen et al., 1980). Thomson and Augusteyn (1983) reported that when the isolation is performed at 37°C, α-crystallin exhibits a molecular weight of approximately 360kDa (S_{20,w} ~12S, called as α_m-crystallin). However, van den Oeterlaar et al., (1985) showed that α-crystallin exists as large aggregate of 800kDa under physiological conditions at 37°C. The aggregation state of α-crystallin and hence the sedimentation coefficient and molecular weight also varies with the experimental conditions such as ionic strength, temperature and pH (Tardieu et al., 1986; Siezen et al., 1980). We have investigated the temperature induced changes in the secondary, tertiary and quaternary structures of α-crystallin (Raman and Rao, 1997) and the results are presented in Chapter III. It is evident from these studies that α-crystallin undergoes structural alterations at elevated temperatures but does not lose its structure.

1.3.5. Molecular chaperone-like activity of α-crystallin

As discussed above, α-crystallin exhibits sequence and structural homology with HSPs. It is expressed constitutively and under stress conditions in non-lenticular tissues as well. Horwitz (1992) showed that α-crystallin prevents the aggregation of other crystallins
and that of several enzymes like a molecular chaperone (A brief introduction to molecular chaperone is given in section 1.4). Consequently, several groups including our own are investigating the chaperone-like property of α-crystallin in order to understand the molecular mechanism and its relevance to the eye lens transparency and cataract. Figure 1.1 demonstrates the chaperone-like activity of α-crystallin towards the heat-induced aggregation of β-crystallin. When a sample of β-crystallin is incubated at 60°C, it aggregates and scatters light which can be measured at an angle of 90° using a fluorescence spectrophotometer. α-Crystallin completely prevents the aggregation of β-crystallin as shown in the figure.

1.4. Molecular Chaperones

In order to fold in the cell, the newly synthesized polypeptide chain has to negotiate an environment far more complex than that faced by the unfolded peptide chain in vitro. The cellular concentration of nascent chains can be very high (estimated to be at least 50μM (Darnell et al., 1986; Hartl et al., 1992)). Most proteins aggregate during refolding in vitro at much lower concentrations than those likely to be found in vivo. Many proteins synthesized in the eukaryotic cell have to be transported across the membranes of organelles such as mitochondria or the endoplasmic reticulum, and are allowed to fold only after they have reached their final destination. As a protein folds in the cell, it may also be subject to various processing events, important both for its function and in determining its extra- or intracellular localization. The protein folding in vivo must, therefore, be controlled or regulated to ensure its efficiency. It has now become clear that such regulation in protein folding is achieved throughout the cell by families of proteins known as molecular chaperones and also
Figure 1.1: Chaperone-like activity of α-crystallin. Aggregation of βL-crystallin (0.2 mg/ml) in the absence (——) and in the presence (---------------) of α-crystallin (0.2 mg/ml) upon incubation at 60 °C. Scattering of samples at 475 nm was monitored in a Hitachi F-4000 fluorescence spectrophotometer. Buffer = 10 mM sodium phosphate (pH 7.4) containing 100 mM NaCl.
by enzymes such as protein disulfide isomerase and peptidyl prolyl cis-trans isomerase.

Many molecular chaperones are also stress proteins. Stress proteins are expressed in increased amounts under stress conditions such as a rise in environmental temperature, and are thought to play a protective and/or restorative role in these processes (Parsell and Lindquist, 1993; Gething and Sambrook, 1992; Pinto et al., 1991). The most predominant heat-induced members of this family are Hsp60, Hsp70, Hsp90, Hsp100 and small Hsps (sHsps; Mol. mass ranging from 15-30kDa) (Parsell and Lindquist, 1993). Many of these proteins are constitutively expressed in low level and are essential for normal growth. Their expression is increased under stress. The exact function of these proteins under stress is not completely understood.

Molecular chaperones are key mediators of protein folding in the cell under normal growth conditions as well as under stress. Chaperones can be involved in the following diverse array of functions in the life cycle of a substrate protein: (i) in cellular protein folding and assembly (see reviews, Agard, 1993; Hartl, 1996), (ii) translocation of polypeptides across membranes and the conformational regulation of signal transduction (chaperone-mediated conformational changes regulate certain signal transduction pathways) (see reviews, Goloubinoff et al., 1989; Hendrick and Hartl, 1993; Clarke, 1996; Hartl, 1996) and (iii) in protein degradation (protein degradation appears to rely on chaperones for the presentation of substrate proteins to proteases in a soluble, degradation-competent state (Hayes and Dice, 1996)).

The mechanism of chaperone action of members of Hsp70 (e.g., bacterial Dnak) and Hsp60 (bacterial GroEL) has been well studied (see review, Martin and Hartl, 1997). Their
high resolution x-ray crystallographic structures are also available (Flaherty et al., 1990; Braig et al., 1994). Chaperones generally recognize the non-native states of many different polypeptides, primarily by binding to solvent-exposed hydrophobic amino acid residues or surfaces. These interactions serve to prevent or reverse off-pathway folding reactions that lead to aggregation. The formation and dissociation of chaperone-substrate complexes is often controlled by ATP binding and hydrolysis by the chaperones, as well as by so-called "co-chaperone" proteins such as Dna J, GroES etc.

Studies on the import of proteins into mitochondria and into the endoplasmic reticulum (ER) demonstrate that different chaperone systems cooperate in the folding of a protein (see reviews, Craig, 1993; Hohfeld and Hartl, 1994; Frydman and Hohfeld, 1997). Following their synthesis in the cytosol, mitochondrial precursor proteins are translocated across the mitochondrial membranes in an unfolded conformation stabilized by cytosolic chaperones. As the translocating polypeptide chain emerges into the matrix of the organelle, it is stabilized by an interaction with a mitochondrial member of the 70kDa heat-shock protein family called mHsp70 which also provides the driving force for translocation (Stuart et al., 1994; Rassow et al., 1995). The folding polypeptide is subsequently transferred to the mitochondrial Hsp60 in the matrix. Hsp60 forms a barrel-shaped double ring structure characteristic for a family of chaperones termed chaperonins, comprising, in addition, bacterial GroEL and the eukaryotic TRiC complex (see review, Hartl, 1996). Together with its regulatory cofactor, Hsp10, Hsp60 appears to provide a sequestered compartment for efficient folding of the imported polypeptide chain (see review, Hartl, 1996). A similar cooperation between an Hsp70 homolog and a cylindrical chaperonin was also observed in chloroplast (see review, Hohfeld and Hartl, 1994).
Translocation of proteins into ER is promoted by the binding of BiP, an Hsp70 homolog of the ER lumen, to the translocating polypeptide chain (Brodsky, 1996). Unlike mitochondria and chloroplasts, the ER lacks a double ring chaperonin. Instead, Bip cooperates with other chaperone systems like GRP94, an ER chaperone homologous to cytosolic Hsp90 (Jakob and Buchner, 1994). Many secreted glycoproteins follow an alternative pathway and interact with calnexin after initial binding to BiP (Bergeron et al., 1994).

The following may be the important determinants of the interaction of a polypeptide substrate with a subset of molecular chaperones on a pathway of protein biogenesis (Frydman and Hohfeld, 1997). (i) The spatial availability of individual chaperone proteins. This depends not only on their subcellular localization and on their expression level, but is also influenced by specific recruitment processes. (ii) The binding specificities of individual chaperone proteins. For example, Hsp70s interact with polypeptides in an extended conformation by recognizing 7-9mer peptide segments with a net hydrophobic character. By contrast, the chaperonins preferentially bind more-structured folding intermediates such as the 'molten-globules'. Structural features sequentially exposed by the folding polypeptide might thus trigger successive interaction with different chaperone systems.

Considerable information is now available on the mechanistic aspects of the chaperone function of Hsp70 and Hsp60 families of proteins. However, such details for other Hsp families such as Hsp90, Hsp100 and small Hsps (including α-crysallin) are still not available. It is known that the members of the Hsp90, Hsp100 and small Hsps are important in preventing protein aggregation under stress (Schirmer et al., 1996; Jakob and
Buchner, 1994). These proteins have been shown to form stable complexes with partially unfolded proteins and prevent aggregation. The more general role of Hsp90 system may be to prevent misfolding and aggregation of pre-existing proteins under stress conditions (Jakob and Buchner, 1994). Hsp90 is found to stabilize guanidinium chloride-denatured β-galactosidase in a soluble state which is competent for subsequent refolding by Hsp70 and Hsp40 during an ATP-dependent reaction (Freeman and Morimoto, 1996). Further studies are expected to provide more mechanistic details of the chaperone functions of these heat shock proteins including the small Hsps like α-crystallin.

1.5. **Scope of the present study**

We set out to investigate the molecular basis of the chaperone-like activity of α-crystallin, its relevance in maintaining the lens transparency and its loss in cataract. Our preliminary observation pointed out that α-crystallin behaves differently towards thermal and non-thermal aggregation of proteins. This led us to use non-thermal mode of aggregation of proteins to study the chaperone-like activity of α-crystallin.

UV light has been shown to be one of the major risk factor in the development of cataract. Among the mammalian lens crystallins, the 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 investigated the chaperone-like activity of α-crystallin towards the photo-aggregation of γ-crystallin. We have also investigated the chaperone-like activity of α-crystallin towards the aggregation of insulin upon breaking the interchain disulfide bonds and rapid refolding-induced aggregation of β-
crystallin. Our studies showed that α-crystallin's ability to function as chaperone is temperature-dependent. A significant enhancement in the activity is seen above 30°C. In order to understand the molecular basis for temperature-dependent enhancement of the chaperone-like activity of α-crystallin, we have investigated the temperature-induced structural changes in α-crystallin by circular dichroism, fluorescence, analytical ultracentrifugation and gel filtration chromatography. We have also investigated the rapid refolding of α-, β- and γ- crystallins in the context of chaperone-like activity of α-crystallin. All our results led us to propose that α-crystallin offers hydrophobic surfaces for partially denatured target proteins and prevents their aggregation. Structural perturbation above 30°C leads to substantial enhancement of exposed hydrophobic surface on α-crystallin and increased chaperone-like activity. It is also possible to perturb the structure of α-crystallin by non-thermal modes to enhance the activity of α-crystallin.

To understand the molecular mechanism of chaperone function, it is important to study the conformational aspects of target proteins which are recognized by α-crystallin. Our investigation on refolding of lysozyme and RNase A and the effect of α-crystallin on the refolding process, provides an insight into the conformational states of refolding intermediate which can interact with α-crystallin.