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α-Crystallin: chaperoning and aggregation

The recent paper by Takemoto et al. (1993) on the chaperoning ability of eye lens α-crystallin shows that the C-terminal region of the protein may be functionally important in protecting proteins from heat-induced aggregation, and so may play a role in chaperoning protein folding. This is significant, as protein unfolding, caused by post-translational modification (Harding and Crabbe, 1984, 1992) or oxidative damage (Wolff and Crabbe, 1985; Wolff and Dean, 1987; Crabbe, 1991) plays an aetiological role in the development of cataract, the largest cause of blindness in the world. Also, α-crystallins have been identified in a large number of extra-lenticular tissues, including heart, brain, spinal cord, liver and kidney (Iwaki et al., 1989), and may act as more general mammalian chaperones.

α-Crystallin exists as mixed multimers of αA and αB peptides; although the C-terminal region is susceptible to post-translational modification (Takemoto et al., 1990), the major site of carbamoylation is near the N-terminus (residue 11 in αA; Qin et al., 1992), and interactions leading to aggregation of subunits are mainly located in the N-terminal half of the chain (Merck et al., 1992). Formation of aggregates is clearly necessary for chaperoning activity. The longest continuous section of homologous sequence between the αA and αB chains lies towards the N-terminus (van der Ouderaa et al., 1974); it contains a largely hydrophobic phenylalanine-rich sequence, RLFDQFF. This is also the longest continuous section of largely hydrophobic sequence in either chain.

The α-crystallin peptides show 40% sequence similarity with small heat-shock proteins (hsps) such as hsp27 (Ingolia and Craig, 1982; Lindquist and Craig, 1988); these hsps also act as chaperones (Ellis and van der Vies, 1991). α-Crystallin can be induced by heat shock (Klemenz et al., 1991), and a heat-shock consensus sequence has been reported for the α-crystallin promoter (Dubin et al., 1990). The interactions between chaperone peptides and between chaperones and unfolded peptides are non-covalent, and are thought to be hydrophobic in nature.

To investigate whether the RLFDQFF sequence was also present in hsps, the programs BLASTP and GAP (University of Wisconsin Genetics Computer Group) were used to compare this sequence with protein sequences in the databases PIR1, PIR2, PIR3 and SWISSPROT, accessed via the SEQNET facility at Daresbury. When sequences of 65–70% similarity and above were allowed, the major proteins which showed sequence similarity over this region were hsps, as shown in Table 1. It therefore seems not unreasonable to suggest that this hydrophobic-rich sequence, which in α-crystallin is probably derived from a primordial hsp gene (de Jong and Hendriks, 1986), may be important in oligomeric interactions in chaperone proteins.

Interestingly, a model structure of the peptide-binding domain of hsp 70 has been based on sequence similarity with the peptide-binding domain of HLA-A2 (Rippmann et al., 1991). Sequence analysis using the GAP program shows a 42% similarity between the bovine αA chain and residues 76–266 of the HLA-A2 α-chain. If one uses this information together with secondary-structure predictions to build a model for the αA chain based on the three-dimensional structure of HLA-A2 (obtained from the Brookhaven database at Daresbury), it is noteworthy that in this putative structure lysine-11, the most susceptible residue in the protein to carbamoylation (Qin et al., 1992), would lie at an exposed turn. Carbamoylation is the post-translational modification resulting from life-threatening diarrhoea, which is a significant risk factor for cataract in tropical countries (Harding, 1985, 1991).

Table 1 Heat-shock-protein sequence similarities to the phenylalanine-rich sequence of α-crystallin

Numbers indicate the residue numbers showing similarity in the different proteins.

^{21}R L F D Q F F^{27}	α-Crystallin
^{28}R L F D Q A F^{34}	hsp 27K hamster
^{28}R L F D Q A F^{34}	Stress protein 25K mouse
^{70}R N F Q Q F F^{77}	hsp htr *Escherichia coli*
^{25}R L F D Q S F^{33}	hsp 25K protein 1AP chicken
^{27}R L F D Q A F^{33}	hsp 27 human
^{109}R L F D D A V^{115}	hsp 26.6K wheat
^{626}K L F E Q L Y^{632}	hsp 70-related protein SSC1 precursor yeast
^{421}K F Y D Q F^{426}	hsp 82K fruit fly
^{23}R L L D D F^{28}	hsp 30 *Neurospora crassa*
^{290}F K Q F F^{294}	Yeast hsp np11

Thus, although the C-terminal region of α-crystallin is clearly important for the protective effect of the protein, this may be because it is this region which is important for the stability of its folded conformation, as it is for many proteins (Creighton, 1993), whereas the N-terminal region contains the hydrophobic site important for aggregate formation.

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