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TRUNCATION OF α A-CRYSTALLIN: EVIDENCE FOR A NEW MODEL OF SMALL HEAT SHOCK PROTEIN FUNCTION

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The small heat shock protein, α -crystallin, plays a key role in maintaining lens transparency by chaperoning structurally compromised proteins. This is of particular importance in the human lens, where proteins are exposed to post-translational modifications over the individual's lifetime. One particular modification of the A subunit of α -crystallin (αA_{WT}), involving the truncation of five C-terminal residues, is found only in the water insoluble fraction of human lens extracts. Previous studies have determined that this truncation has no effect upon the chaperone efficacy of αA_{WT} , however its interaction with full length α -crystallin has not been explored. Here, we have prepared a five residue truncation mutant (αA_{1-168}), and examined its structural and functional characteristics using nano-electrospray mass spectrometry performed on a Q-ToF 2 instrument (Micromass UK Ltd) which has been modified for high mass operation. αA_{1-168} was found to have an average oligomeric molecular weight of approximately 100 kDa less than αA_{WT} . Associated with this decreased size, a slight reduction in polydispersity, and an increased preference to form even-numbered oligomers was also observed. A novel MS/MS method was developed to examine the kinetics of subunit exchange with αA_{WT} and αB_{WT} . The data revealed that there was a 2-3 fold decrease in the rate of exchange when αA_{1-168} was involved. Furthermore, exchange between the wild type proteins revealed that αA_{WT} subunits formed a core within the mixed assemblies. The reduced exchange kinetics of αA_{1-168} had no effect upon chaperone efficiency, implying that the rate of subunit exchange in α -crystallin oligomers is not the critical parameter in efficient chaperone behavior. These results suggest a profoundly different mechanism of small heat shock protein chaperone behavior between mammals and other species.