ANALYSIS OF THE FUNCTIONAL ROLE OF A REGION IN THE ALPHA-CORE DOMAIN OF SMALL HEAT SHOCK PROTEINS

by

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ABSTRACT

αA-crystallin is the major protein component of the mammalian lens, where it functions to provide the additional refractive power and maintain the clarity necessary for lens function in vision. It is also an important member of the small heat shock protein (sHSP) super-family, exhibiting chaperone-like activity, the ability to prevent the super-aggregation of denatured or denaturing proteins.

The crystallization of αA-crystallin is not achieved yet. The crystallography of two members of plant/bacterial sHSPs, a mammalian co-chaperone, p23, and a sHSP from beef tapeworm, are available. All these sHSPs have a highly conserved α-core region. However, they also have some major differences. First, Hsp16.5, the representative of plant/bacterial sHSPs, is highly symmetrical and forms a hollow sphere with exactly 24 subunits, while αA-crystallin is proposed to have an irregular shape and aggregate size. Second, sequence analysis among different sHSPs revealed that the loop of αA-crystallin equivalent to the dimerization loop in Hsp16.5 is much shorter and lacks the β-sheet sequence, and this region in p23, which is monomeric, is only 4 amino acids long. The loop region of αA-crystallin is not long enough to have the same function to control dimerization and α-core region orientation. The aims of this study were to determine the functions of the loop region in αA-crystallin. Sets of loop-deleted or swapped chimeras were constructed in both Hsp16.5 and αA–crystallin, and analyzed with several biophysical and biochemical methods. The different effects of these deletions or replacements of the loop region on different species of sHSPs allowed us to detect whether any change in the protein structure, stability and function occurred due to the mutation. Results from this study suggest that the deletion or replacement of the loop region in αA-crystallin has different effects than the same change in Hsp16.5. Hsp16.5 and αA-crystallin might have different mechanisms to assembling their subunits to form high order aggregates. The change of the loop region in αA-crystallin affects the protein's aggregation size, stability, and chaperone-like activity. The results from this study provide a foundation for further studies on the structure and function of αA-crystallin or other mammalian sHSPs.