Recovery of Argininosuccinate Lyase Activity in Duck δ1Crystallin

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δ-Crystallin is directly related to argininosuccinate lyase (ASL). Two isoforms exist in ducks, δ2 and δ1, which are 94% identical. δ2 is the duck orthologue of ASL, while δ1 is enzymatically inactive. Chimeras of the two isoforms have shown that domain 1 of δ2 is sufficient to recover activity in δ1. Structural comparisons of various δ-crystallin proteins revealed that conformational differences between δ1 and δ2 are localized to residues 23-32 and 74-89 (20's and 70's loops). As the putative catalytic residues are conserved in δ1, the amino acid substitutions in these loops are thought to prevent substrate binding in δ1. However, a δ1 double loop mutant (DLM), with all residues in the 20's and 70's loops replaced with those of δ2, was found to be inactive and binding of the substrate to the DLM could not be detected by ITC. To further investigate this result, crystal structures of the DLM with and without sulfate bound have been determined to 2.2 and 2.5Å resolution, respectively. The conformations of the 20's and 70's loops in the DLM and δ2 are very similar, suggesting the remaining five amino acid differences in domain 1 of the DLM relative to δ2 are important for ASL activity. Mutagenesis experiments reveal that ASL activity can be recovered in the DLM by mutating Met-9 to Trp. Truncation mutants of δ2 demonstrate that although the N-terminal arm is conformationally flexible, this region of the protein is critical for ASL activity. The N-terminal segment is likely involved in stabilizing regions of δ2 involved in substrate binding and catalysis.

Keywords: δ-crystallin, argininosuccinate lyase, enzyme mechanism