

3D Domain-swapped dimers of human cystatin C and their interactions - implications for amyloidogenesis. M.Jaskolski, R.Janowski, *Department of Crystallography, A.Mickiewicz University and Center for Biocrystallographic Research, Inst. Bioorg. Chem., Pol. Acad. Sci., Poznan, Poland*

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Proposed earlier as a possible mechanism of amyloid formation, the phenomenon of 3D domain swapping has been first observed in aggregation of an amyloidogenic protein in the case of human cystatin C (HCC). HCC is a physiological inhibitor of cysteine proteases, present in particularly high concentration in the cerebrospinal fluid, but it is also found in amyloid aggregates formed in the brain arteries at advanced age. This pathological process is greatly accelerated with a naturally occurring L68Q variant, resulting in fatal amyloidosis in early adult life. When proteins are extracted from HCC amyloid deposits, an N-truncated cystatin C variant (THCC) is found, lacking the first 10 amino acids of the native sequence. Human cystatin C has been studied in three crystal forms, one of which corresponds to the N-truncated variant, and in all cases 3D domain-swapped dimers were found. The dimers arise by partial unfolding of the monomeric protein through opening of a β -hairpin loop followed by a reconstruction of the monomeric fold from two protein chains. The domain that is exchanged consists of a long α -helix flanked by two β -strands. In one of the structures, the dimers have crystallographic two-fold symmetry, while in all other cases (involving multiple copies in the asymmetric unit) they are pseudosymmetric. The act of domain swapping is in all the cases the same (conservation of the closed interface) but the dimers are not identical, reflecting the flexibility of the linker region (open interface). This is particularly pronounced in the newly determined tetragonal structure, where the mutual orientation of the two monomer-like domains is totally different from the previous situations. The closed dimers enter into conserved intermolecular interactions suggestive of their possible significance for higher aggregation of HCC. One of the packing modes involves the formation of closed octameric assemblies via convoluted H-bonding interactions between a loop structure and the extended molecular β -sheet. Another pattern involves hydrophobic interactions between the dimer-specific open interface regions. The most intriguing patterns take the form of intermolecular β -sheet bonds, reminiscent of the cross- β interactions believed to form the core of the amyloid structure. In the case of THCC, a dimer β -sheet is extended to another dimer with recruitment of a water molecule. The tetragonal HCC structure suggests how the β -sheet interactions could be propagated infinitely, as would be expected in an amyloid fibril.