Proline Isomerization in Stefin B: a Crucial Step Towards Amyloid Fibril Formation

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For prion proteins as well as cystatins it has been suggested that formation of the 3-dimensional domain swapped dimers is the crucial step in fibril formation process, whereas higher order oligomers have not been characterized so far. One of the mutants of stefin B, the P79S, exhibited a higher stability and a prolonged lag phase of fibrillation. It forms tetrameric oligomers, which we were able to crystallize and determine their structure at 1.4 Å resolution (a=120Å, b=31Å, c=51Å, $\alpha=\gamma=90^{o}$, $\beta=96^{o}$, space group C2). The tetramer structure is built from a pair of domain-swapped dimers related by a crystallographic 2-fold axis. The structure comparison with the native stefin B structure revealed that the flip of the Ser72-Leu80 loop is associated with the trans to cis isomerization of the peptide bond of Pro74, which is the only absolutely conserved proline residue in the cystatin family of the cysteine protease inhibitors. The crucial role of the proline peptide bond trans-cis isomerization is further supported by the activation energy needed for stefin B P79S mutant to undergo tetramerization,, which corresponds to the energy of proline isomerization. These data suggest that the proline isomerization may be the crucial step governing the kinetics of stefin fibril growth.

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