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Pressure as a denaturing agent in studies of single-point mutants of an amyloidogenic protein human cystatin C.

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Streszczenie

Recently, we presented a convenient method combining a deuterium-hydrogen exchange and electrospray mass spectrometry for studying high-pressure denaturation of proteins (Stefanowicz et al., Biosci Rep 2009; 30:91-99). Here, we present results of pressure-induced denaturation studies of an amyloidogenic protein-the wild-type human cystatin C (hCC) and its single-point mutants, in which Val57 residue from the hinge region was substituted by Asn, Asp or Pro, respectively. The place of mutation and the substituting residues were chosen mainly on a basis of theoretical calculations. Observation of H/D isotopic exchange proceeding during pressure induced unfolding and subsequent refolding allowed us to detect differences in the proteins stability and folding dynamics. On the basis of the obtained results we can conclude that proline residue at the hinge region makes cystatin C structure more flexible and dynamic, what probably facilitates the dimerization process of this hCC variant. Polar asparagine does not influence stability of hCC conformation significantly, whereas charged aspartic acid in 57 position makes the protein structure slightly more prone to unfolding. Our experiments also point out pressure denaturation as a valuable supplementary method in denaturation studies of mutated proteins.

Słowa kluczowe

unfolding/refolding, hydrogen deuterium exchange, mass spectrometry, electrospray ionization

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