STRUCTURAL STUDIES OF COVALENTLY STABILISED OLIGOMERS OF HUMAN CYSTATIN C

M. Kozak[1*], **M. Taube**[1], **M. Murawska**[1], **V. Lindström**[2], **and A. Grubb**[2]

[1] *Department of Macromolecular Physics, A. Mickiewicz University, Poznan, Poland*
[2] *Department of Clinical Chemistry and Pharmacology, Lund University Hospital Lund, Sweden*

Keywords: synchrotron radiation, cystatin, amyloid
[*] *e-mail*: mkozak@amu.edu.pl

Human cystatin C (HCC) is a small protein (MW 13.4 kDa), acting as an inhibitor of cysteine proteases. In pathological conditions HCC undergoes oligomerisation via the domain swapping mechanism. Oligomerization of HCC leads to formation of amyloid deposits in brain arteries [1]. This protein was also found as codeposits in the amyloid plaques of Alzheimer's disease or Down's syndrome. So far two crystal structures of full length (native) dimeric forms of HCC and monomer-stabilized with an engineered disulfide bond (Leu47Cys)-(Gly69Cys) have been solved by X-ray crystallography [2]-[4].

The aim of this study was the characterization of native protein and covalently stabilized monomers and oligomers (dimers, trimers, dodecamers and icosatetramers) of human cystatin C in solution using small angle X-ray scattering (SAXS).

Combination of synchrotron radiation and SAXS method in structural analysis of macromolecules in solution enables not only determination of the basic structural parameters (the radius of gyration, the maximum size of particle or molecular weight) but also permits a verification of the crystal structure of biomacromolecules with the scattering data collected in solution and provides information on the possible conformational changes taking place in solution. Detailed analysis of SAXS data including the *ab initio* shape-determination algorithms provides also the information on the envelope (the shape) of the protein molecules in solution.

The SAXS data were obtained using synchrotron radiation in MAXLab (beam line 9-11-4, MAXII storage ring) and DESY (beam line X33). Using shape determination program DAMMIN [5] the low resolution models in solution of native HCC and covalently stabilized HCC oligomers (monomer, dimer and trimer). Native monomeric HCC irradiated by synchrotron radiation undergoes dimerisation in solution. Dimeric native HCC form in solution a dimer with elongated shape. The conformations of HCC molecules (monomeric and dimeric forms) in solution were evaluated by the use of CRYSOL [6]. Monomeric HCC and both dimeric crystal structures (cubic and tetragonal) were compared with SAXS data. This comparison clearly indicated that the preferred conformation of native dimeric HCC occurring in solution is the conformation of extended dimer observed in the tetragonal form.

Also the processes of formation of oligomers of native HCC in different pH and temperature conditions were monitored using SAXS in 1h time steps. The structures of HCC oligomers were also characterized by microscopic methods.

Acknowledgments: This research was supported in part by research grant (No N N202 127237) from the Ministry of Science and Higher Education (Poland). The data collection was supported by European Community — EMBL Hamburg Outstation, contract number: RII3-CT-2004-506008.

References

[1] A.O. Grubb, "Cystatin C — properties and use as diagnostic marker," *Adv. Clin. Chem.* **35** (2000) 63 - 99.

[2] R. Janowski, M. Kozak, E. Jankowska, Z. Grzonka, A. Grubb, M. Abrahamson, M. Jaskolski, "Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping," *Nature Struct. Biol.* **8** (2001) 316 – 320.

[3] R. Janowski, M. Kozak, A. Grubb, M. Abrahamson, M. Jaskolski, "3D domain-swapped human cystatin C with amyloidlike intermolecular beta-sheets," *Proteins: Struct. Funct. Bioinf.* **61** (2005) 570 – 578.

[4] R. Kolodziejczyk, K. Michalska, A. Hernandez-Santoyo, M. Wahlbom, A. Grubb, M. Jaskolski, "Crystal structure of human cystatin C stabilized against amyloid formation," *FEBS J.* **277** (2010) 1726 – 1737.

[5] D.I. Svergun, "Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing," *Biophys. J.* **76** (1999) 2879 – 2886.

[6] D. Svergun, C. Barberato, M.H.J. Koch, "CRYSOL — a program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates," *J. Appl. Cryst.* **28** (1995) 768 – 773.