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Preliminary results of human PrP^C protein examination by spectroscopic techniques

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There are almost 90 proteins that are considered to be potential amyloidegenic factors [1]. One of them, prion protein (PrP) is natural glycoprotein which occurs on surface of cell membranes. It has been shown that PrP protein is able to transform itself in post-translation conversion from native cellular (PrP^C) state to pathological, proteinase resisted amyloid (PrP^{Sc}) form. Such conformation alteration of PrP is directly linked to non-curable transmissible spongiform encephalopathies (TSE) diseases.

Little is known about role of native PrP^C protein in cell activity, however it was proven that it is able to bind Cu^{2+} ions [2] as well as other metal ions on 2+ oxidation state. 3D structure of PrP^C protein contains two domains, N-terminal part, composed of 102 amino acid residues (residues: 23-124), is presented as unstructured domain which has ability to coordinate Cu^{2+} ions mostly through a conservative fragment consisting four tandem repeats of the PHGGGWGQ sequence [3]. Amount of bonded ions varies with ion concentration in solution. Second C-terminal domain (residues 128 - 231) has globular fold built with three α–helices and two antiparallel β-sheets [4]. PrP^C protein has already been examined using various methods including SR-SAXS spectroscopy on purified amyloid plaques [4] and biochemical approaches. Structure of Cu-binding sites remains unknown due to high mobility of unstructured domain which provide to unambiguous results. However, application of X-ray absorption spectroscopy supported by other spectroscopic methods have to deliver data about coordination of Ci(II) binding site.

Main research objective is to obtain structural information about coordination of Cu(II) in PrP^C protein. Our project request detail analyze of coordination environment and structures of both native and mutated PrP^C forms and with Cu^{2+} ion substituted by Zn^{2+}.

In the initial stage, the PrP^C purifying protocol of obtained lyophilized PrP proteins with and without bounded Cu(II) ions has been established. First preliminary XAS measurements have already been carried out on SuperXAS beamline (SLS, PSI Villigen) on both PrP^C-Cu(II) and PrP^C-Zn(II) complexes and results were compared with theoretical predictions using FeFF9.6 [6] software (Figure 1).

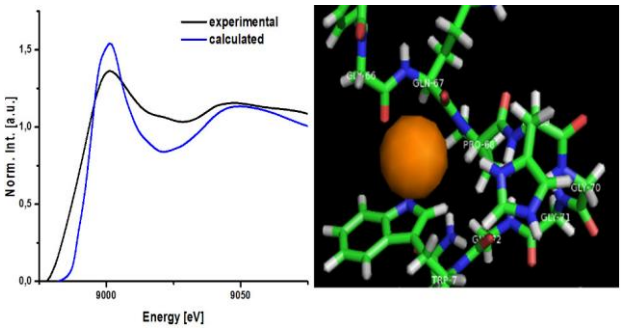

Figure 1. Results for XAS mesurment on PrP^C-Cu complex compared with theoretical predictions based on modified crystallographical structure. (left) smoothed XANES spectrum compared to calculated theoretical one; (right) 3D structure of hypothetical binding site of Cu^{2+} ion (orange sphere). Structure was taken from PDB database, PDB ID: 2kkg and modified manually.

Complementary to XAS data, AFM measurements have been performed to obtain general structural information about sample and to establish a protocol of fixing protein molecules on solid substrate in order to perform next step of experiments. It has been found out that C-terminal domain of PrP^C molecule has around 5 nm in diameter.

Presented results reveals that both XAS and AFM methods are promising tools in further examination of human PrP^C protein and its mutants. However more spectroscopic techniques have to be applied in order to have complex view of studied system.

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