Attempt to determine the structure of the huPrP^{C}-Cu(II)/Zn(II) complexes.

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*hu*PrP^{C} (human Protease Resistant Protein) is membrane protein that has two main structural domains. C-terminal globular domain anchors whole protein in lipid membrane, whereas N-terminal domain remains unstructured above the membrane [1]. The unstructured part has high affinity to bind divalent metals, especially to Cu(II) and Zn(II). In posttranslational conversion process the cellular (PrP^{C}) form transforms into pathologic *scrapie* form (PrP^{Sc}) that can form amyloidogenic deposits in nervous cells which are resistant to proteinase enzymes. While the structure of both PrP^{C} and PrP^{Sc} as well as disease progression are well known, the process of PrP^{C}→PrP^{Sc} conversion and the role of metal ions binding are poorly understood. Presented work shows one of the first spectroscopic results for wild-type human PrP^{C} protein (23-231) complexed with Cu(II) ions.

Cu K edge XAS experiments were done at SuperXAS beamline in Paul Scherrer Institut (PSI) (Villigen, Switzerland) and PrP^{C}-Cu(II) protein (23-231) complex solutions of 50 μM were measured in Kapton tubes at room temperature. Next set of XAS Cu K edge measurements were conducted at XAFCA beamline in Singapore Synchrotron Light Source (Singapore) [2] on lyophilized PrP^{C}-Cu(II) protein (23-231) complex at room temperature. Complex was in form of pellet mixed with BN. Finally, Cu and Zn K edge XAS experiments were performed at P64 beamline in DESY (Hamburg, Germany) on lyophilized PrP^{C}-Cu(II) (23-231) and PrP^{C}-Zn(II) protein (58-93) complexes at temperature of 10 K. Data analysis was performed using Demeter [3] package while for *ab-initio* calculations FEFF [4], FDMNES [5] and Orca [5] software were used.

Spectra obtained from PrP^{C}-Cu(II) complex in water solution and as powder sample were different especially in pre-edge region. There was very wide (~22.2 eV) pre-edge structure for spectrum obtained at 10 K proving strong orbital mixing, while in case of water solution pre-edge feature was much sharper (FWHM~4.6 eV). It was shown that, in case of solution, local geometry of Cu(II) ion is linear combination of two biding modes. First consist of 3 nitrogen atoms and one oxygen atom while the second one is composed from 4 nitrogen atoms. This was in agreement with our previous study and literature reports [6],[7]. In case of PrP^{C}-Zn(II) complex due to its higher stability, more binding modes were detected . Increase PrP^{C} : Zn(II) ratio from 1 : 1 up to 1 : 10 resulted in domination of 3N – O binding mode. Therefore there was a competitive mechanism of Zn(II) binding. In cases of both Cu(II) and Zn(II) ions coordination environment, Histidine residues influence were evidenced and all binding sites were mononuclear.

Membrane protein such as PrP^{C} are very difficult subject of structural study. We have shown that change of solution can alter local coordination geometry of Cu(II). Moreover, we have proven alteration of binding site structure under increase concentration of Cu(II) or Zn(II) ions. This may be important factor to understand pathologic $PrP^{C} \rightarrow PrP^{Sc}$ transformation however many questions still need to be answered in this matter.

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