The Zn2+ -dependent change of Nucleobindin-2 structure

P-02.5-49

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Nucleobindin-2 (Nucb2) is a physiologically relevant protein, which participates in variety of biological processes i.e. carcinogenesis, food intake inhibition and sleep regulation. Additionally, broad expression level of Nucb2 has been found in central nervous system, adipose tissue, heart, pancreas and digestive system suggesting its potentially new roles in various metabolic functions. Metal ions such as Ca^{2+} and Zn^{2+} might modulate the structure of proteins to facilitate performing their functions. Nucb2 contains the C-terminus two EF-hands, responsible for Ca^{2+} binding and a putative Zn^{2+}-binding site at the N-terminus. Two homologs, Nucb2 from *Gallus gallus* (ggNucb2) and *Homo sapiens* (hsNucb2) was previously characterized as Ca^{2+}-binding intrinsically disordered proteins (IDPs). The aim of our study was to investigate if and in which way Nucb2s structure is modulated by Zn^{2+} ions. We prepared both homogeneous proteins in a four step procedure: ion metal affinity chromatography (IMAC), digestion of the His-Tag by HRV 3C protease, second IMAC and gel filtration. The hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) showed that peptides at N-terminal half of Nucb2s, located in the near proximity of the putative Zn^{2+} binding site, are more solvent exposed in the presence of Zn^{2+} than in the absence of these ions, which suggests that this part of the protein is characterized by a significant flexibility. Simultaneously, the Trp fluorescence spectra revealed that Nucb2s undergo compaction upon Zn^{2+} addition. Surprisingly, two Trp residues of Nucb2s are located at C-terminal half of proteins. We concluded that the Zn^{2+} impacts both parts of Nucb2s, leading to the structural rearrangement of the entire protein molecule. The functional importance of these alternation should be further explained.

Funding: This work was supported by the National Science Centre Grant (A.O.) 2018/29/B/NZ1/02574.