Study of NdCTR1 properties and its purification method

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Copper (Cu) is a vital trace element as its ions are cofactors for a number of essential enzymes and also directly participate in regulation and signalling. Despite this fact free Cu ions are toxic as they induce ROS formation. Cu dyshomeostasis leads to development of the inherited and sporadic diseases, also Cu is necessary for tumor growth. Chelation therapy is a promising approach in treatment of mentioned disorders but nowadays there's a lack of natural chelators with no side effects. In this work we suggest the 55 a.a. extracellular N-terminal domain of human copper importer CTR1 (NdCTR1) as a safe natural Cu chelator, describe its purification procedure and partly its properties. Initially 67 a.a. NdCTR1 was expressed as a GST-fused protein. GST-NdCTR1 producing bacteria accumulated more Cu and Ag ions (Ag^+ ion is similar to Cu^+) than a control GST-synthesizing strain. Presence of GST-NdCTR1 in E. coli increased their resistance to mentioned ions as well as to Ag nanoparticles due to their chelation by NdCTR1 what was shown by SEC of lysates and GST-NdCTR1 immunoprecipitation with subsequent measurement of metals by AAS in obtained fractions. GST dimeric nature and NdCTR1 hydrophobic amino acid cluster (55-67 a.a.) led to GST-NdCTR1 accumulation in inclusion bodies without possibility of refolding after denaturation by chaotropes. To overcome solubility problem NdCTR1 was fused with GB1 protein which was soluble up to 1 mM. GB1-NdCTR1 was purified by IMAC on Cu-charged NTA-sepharose with subsequent SEC. GB1-NdCTR1 binds Cu and multimerizes upon binding as was shown by Cu accumulation in corresponding molecular weight fraction and spectrophotometry. Pure NdCTR1 was obtained after GB1-NdCTR1 proteolysis with thrombin and IMAC. NdCTR1 chelating properties are being researched at the moment and its use as a chelating agent in therapy of Cu-related diseases is discussed. The work was supported by RFBR grants 19-315-90129, 18-515-7811 and RSF grant 20-74-10087.