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Metallothioneins (MTs) are classified as low molecular weight, cysteine-rich, metal binding proteins. The large number of cysteine (Cys) residues in MTs bind a variety of metals by mercaptide bonds [1]. A novel MT gene (dmt) in Triticum durum was identified and cloned for overexpression in E.coli [2]. T. durum metallothionein (dMT) displays three sequence domains: metal binding N terminus (β domain, 1-19th residues) and C terminus (α domain, 61-75th residues) and a long hinge region (20-60th residues). Cysteines are clustered equally in N and C termini with a "Cys-X-Cys" motif (Cys-motif) and the hinge region possess no Cys residues. dMT was overexpressed in E.coli as a GST (glutathione-S-transferase) fusion protein (GSTdMT). Both GSTdMT and dMT cleaved from GST were purified and characterized by biochemical and biophysical methods. It was shown that GSTdMT binds 4±1 moles of Cd per one mole of protein and has a high tendency to form stable oligomeric structures [3]. The aims of the present work are investigation of the effect of removal of the hinge region connecting the two metal binding domains on the stability of the protein structure, and determination of the effect of Cys-motif modifications on the metal binding capacity and affinity of dMT. Furthermore removal of the hinge region will allow comparison with the structure of mammalian MTs which tend to possess short connecting hinge region. Structural features of all mutants will be investigated using biophysical methods such as gel filtration chromatography, SDS- and native PAGE, dynamic light scattering, atomic absorption spectroscopy and circular dichroism spectrometry. Removal and linking procedure of hinge region are executed by PCR techniques. The "chimeric" dMT is inserted to the vector pEGX4T-2 and BL21 strain E.coli has been transformed with this construct. Cys motifs are modified to produce mutant proteins with CCSCG, GCSCC or CCSCC motifs. Mutations are accomplished by site-directed mutagenesis and the mutant constructs are introduced into the pGEX4T-2 vector for expression in E. coli. Results of mutations on the expression of recombinant proteins and their metal-binding properties will be presented.

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Structural Study of Metal Nanoparticles, Promising Biotechnological Materials. Eleonora Shtykova[a], Petr Konarev[b], Lyudmila Bronstein[c], Dmitri Svergun[b]. [a]*Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia.* [b]*European Molecular Biology Laboratory Hamburg Outstation, Hamburg, Germany.* [c]*Department of Chemistry, Indiana University, USA.*
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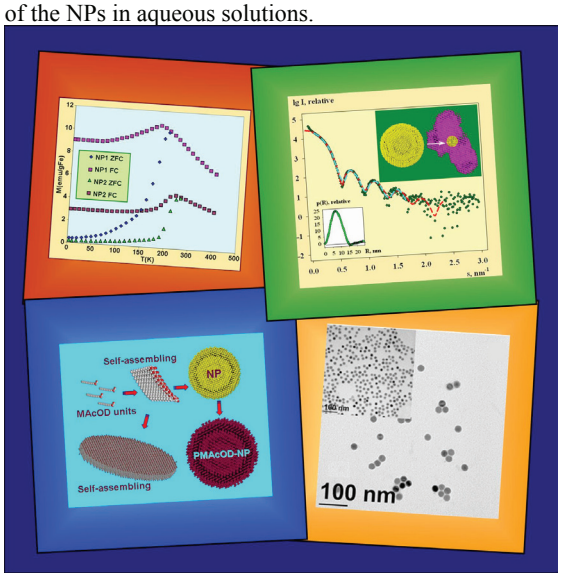
Iron oxide magnetic nanoparticles (NPs) are increasingly popular in life science and medicine because they are easily metabolized or degraded *in vivo*, and can be used as biosensors, bioprobes, in cancer treatment, *etc*. Precondition for such applications is water solubility, which can be achieved by introducing a biocompatible shell on the hydrophobic NP surface. In this work we report structure and properties of iron oxide NPs synthesized by decomposition of iron oleates and encapsulated by different methods. The detailed structure of these particles in aqueous solutions was determined using small angle X-ray scattering (SAXS) providing structural information at resolution from about 1-2 to about 100 nm. The SAXS studies were complemented by several other methods, in particular, X-ray diffraction, transmission electron microscopy, dynamic light scattering, magnetometry etc providing a comprehensive description of the NPs formation and encapsulation processes. Novel SAXS data analysis methods [1-2], in combination with several complementary techniques allowed us to build detailed low resolution three-dimensional structural models of the NPs in aqueous solutions.



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