Abstract

In the work, the selection of conditions for the synthesis of complexes and nanocomposites based on lactoferrin and ions of d-metals of biological importance, namely silver, iron(III), and zinc ions, was carried out. The influence of various factors on the efficiency of metal binding to the protein was investigated. Among others, the influence of pH and concentration of metal ions on the properties of the obtained biologically active substances were examined. The obtained nanocomposites and metal-rich protein complexes were characterized in terms of physicochemical and biological properties. Electron microscopy, spectrometric, spectroscopic, and coupled separation techniques were used for these purposes. The stability of the complexes in buffers imitating the conditions of various parts of the digestive system was also determined. The possibility for the utilization of as-synthesized complexes as active substances in pharmaceutical preparations or dietary supplements was also assessed based on the *in vitro* methods for antibacterial properties and cytotoxicity study. A high potential for the use of the proposed synthesis methods in the pharmaceutical, cosmetics, and food processing industries was found. Finally, an attempt was made to determine the actual mechanism of interaction of metals with proteins on the example of Zn^{2+} binding to human lactoferrin by applying computational methods of molecular dynamics and density functional theory.

The initial stage of the research included the characterization of model proteins, i.e. bovine lactoferrin and recombinant human lactoferrin. The molecular weight, isoelectric point, and the content of iron and zinc ions were determined, which is extremely important for the determination of the processes that take part during the interaction of metals with proteins and the stoichiometry of the reaction. In the next stage, the nature of the process was described using the classical sorption approach. Another important stage of the work was to complement the sorption data with data from spectroscopic methods and electron microscopy. The results of electron microscopy revealed the formation of homogeneous complexes of iron and zinc and a nanocomposite of silver with lactoferrin. Moreover, the dependence of the size of silver nanoparticles on the concentration of silver in the solution was demonstrated. In addition, a correlation was found between the shape of the adsorption isotherm and the size and number of metallic inclusions in the nanocomposite. Spectroscopic studies showed the participation of functional groups in the binding of metals, where the largest share for all the tested metals had the carboxylic groups of glutamic and aspartic acids. Additionally, the participation of functional groups of other amino acids – histidine, tryptophan, serine, tyrosine, and threonine has also been demonstrated. It was also found that the binding of metals to the protein induces changes in its structure and the emergence of new metal-binding sites with a simultaneous change in the iron-

binding capacity. The good agreement between experimental and theoretical results allowed us to propose a mechanism of interaction of zinc with lactoferrin. Finally, it was possible to identify each individual stage of the changes that take place during the metal binding.

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