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Badanie mechanizmów tworzenia nanokompozytów metal-białko jako potencjalnych środków przeciwdrobnoustrojowych

Abstract

In this doctoral dissertation, immobilization of zinc and silver ions to whey protein (*β*-lactoglobulin) and zinc ions to individual casein fractions (α_{S1}-, *β*-, *κ*-CN) was carried out. Mechanisms of binding and sorption capacities of proteins (biocolloids) were investigated using kinetic and isothermal studies, as well as molecular modeling/docking methods and quantum calculations. The obtained nanocomposites were subjected to physicochemical characterization using the whole spectrum of instrumental techniques such as spectrometric, spectroscopic, microscopic and diffraction methods, which complemented each other. Another aspect of the studies, due to the emerging problem of drug resistance and the search for new antiseptics, application studies were performed to determine the antimicrobial potential of the synthesized nanocomposites. The culmination of the research and an extremely important issue undertaken in the dissertation was the use of an animal model (mice) to determine the effect of the obtained nanocomposites on the process of wound healing as well as the distribution of zinc and silver in organs (liver, blood).

The initial stage of the study consisted of characterizing the proteins involved in the binding process using MALDI-TOF MS and determining their isoelectric points by measuring the zeta potential and determining their stability. Furthermore, the results of proteomic methods based on classical protein digestion in solution were compared with microfluidic immobilized enzyme reactor (μ-IMER) using ZipTip pipette tips. Subsequently, the synthesis of metal ions with the studied proteins was carried out by performing classical kinetic and isothermal studies, which allowed us to understand the complex and multistep mechanisms of complex formation. The obtained experimental data were compared with selected kinetic models (zero-order, first-order, pseudo-first-order, pseudo-second-order, intraparticle diffusion Weber-Morris model) and isothermal models (Henry, Freundlich, Langmuir). A whole range of instrumental techniques showing high complementarity among themselves allowed the identification of surface functional groups of proteins involved in the binding process, determination of surface morphology and stability, also with emphasis on the effect of metal concentration. An important step in the work was the confrontation of experimental results with molecular modeling/docking

and quantum mechanical calculations. The high agreement obtained between experimental and theoretical results allowed us to conclude that in the case of interactions of zinc ions with proteins we are dealing only with metallocomplex type systems. In contrast, in the case of interactions of silver ions with βLG, a hybrid system is formed – a metallocomplex with the formation of silver nanoparticles (AgβLG/AgNPsβLG). In addition, antimicrobial activity of Zn/AgβLG, Znα_{S1}CN, ZnβCN, ZnκCN nanocomposites against clinically relevant pathogens was demonstrated. *In vivo* application studies conducted on an animal model (mice) indicate an observable increase in the body weight of rodents after ten-day observation, gradual regeneration of the animal body, the appearance of scab and regrowth of hair. The study shows that metal-protein interactions with antimicrobial properties can contribute to solving the problem of drug resistance and become a potential candidate as a preparation used to treat hard-to-heal wounds.