Tytuł projektu

Badanie molekularnych mechanizmów wiązania metali z białkami

Project title

Study of molecular mechanisms of metal-protein binding

Dyscyplina /Area of science

Nauki chemiczne

PROJECT DESCRIPTION

Project goals

- To study physicochemical, biochemical and microbiological properties of cow's milk biocolloids
- To perform the preparative isolation of milk and whey proteins
- To perform isolation, identification and selection of lactic acid bacteria (LAB)
- To investigate the kinetics of d-electron metal ions binding into/onto protein
- To synthesize metal-protein composites and investigate its physicochemical and biological properties
- To describe metal-proteins molecular mechanism interaction

Outline

Nowadays, silver and zinc are used in medicine in many bactericidal formulations. Ag^{+} and Zn^{2+} ions have high affinity for functional groups such as phosphate, carboxyl, amino, sulfhydryl and imidazole. These groups are part of the proteins and nucleic acids of both prokaryotic and eukaryotic cells. The affinity of Ag^{+} and Zn^{2+} ions to functional groups of the ribosome active site results in the inhibition of translational processes and the blocking of the oxidative enzymes involved in the respiratory chain. This leads to excessive production of free radicals. Free ions interact with the nitrogenous bases in the DNA which leads to the formation of a condensed nucleic acid form. Due to the fact that replication can only undergo DNA in relaxed form, the presence of the Ag^{+} ions blocks bacterial and eukaryotic cell division. Hence, the cytotoxic properties of silver and zinc ions in the human body result. In order to eliminate its cytotoxic effect, it is important to obtain zinc oxide as a biocolloid (e. g. as a complex with bioactive ligands such as proteins) or as a zinc oxide nanoparticles.

One of the promising tool in development of effective antibacterial substances can be reached by the synthesis of complexes composed of metal nanocomposites and whey proteins deposit produced by LAB isolated from whey waste. Hence, the objective of the project is the synthesis of biologically active systems such as:

- metal ions (e.g. silver and zinc) bonded to milk and whey proteins (metal-proteins complexes),
- silver (Ag) and zinc oxide (ZnO) bionanoparticles synthesized by probiotic lactic acid bacteria isolated from dairy products.

The research goal of the project will be to study the mechanism of metal ions binding to protein and the formation of metal/metal oxide nanoparticles as a results of microbiological synthesis. The use of instrumental methods, taking into account molecular changes and surface morphology, will allow to describe the binding phenomena between the metal ion and protein and the formation of metal and their oxide nanoparticles. In addition, the use of quantum-mechanical calculations for interpretive purposes will allow the in-depth analysis of the oxidation-reduction and surface binding processes of metal ions to the active functional groups of protein.

Recently, the antimicrobial mechanism of metals/metal oxides nanoparticles and protein composites has not been fully understood. Hence, it is planned to determine the biological potential and to investigate the antibacterial, antifungal and anti-cancer properties of the resulting metal-protein composites and metal /metal oxide nanoparticles using the most advanced research instrument.

Work plan

1. Physicochemical and biochemical studies of row material - determination of proteins, lipids and volatile organic compound in milk and whey samples and preparative separation of proteins from liquid whey, whey protein concentrate (WPC) and whey powder.

2. Isolation, identification and selection of LAB from milk and whey samples.

3. Synthesis of metal-protein complexes based on milk and whey proteins. Study of metal ions binding to proteins.

4. Biological synthesis of silver and zinc oxide nanoparticles using lactic acid bacteria isolated from milk and whey.

5. Physicochemical characterization of obtained preparations using instrumental methods such as spectroscopy, electron microscopy and spectrometry.

6. Description of mechanisms (metal ions binding to proteins and formulation of nanoparticles) by quantum - mechanical calculations and molecular modeling.

7. Examination of antimicrobial, antitumor and cytotoxic effect of obtained metal-protein complexes and Me/MeObionanoparticles. Study of antibacterial, antifungal and cytotoxicity mechanisms.

8. Studies on the migration of obtained metal nanocomposites and nanoparticles in the pig and mouse skin as well as the wound healing process using murine model

Literature

V. Railean-Plugaru, P. Pomastowski, M. Szultka, B. Buszewski, Analityka (2014) 3, 8–17

P. Pomastowski, M. Sprynskyy, M. Słodowska, B. Buszewski, Przem. Chem. (2016) 95, 1420–142

P.Pomastowski, M. Sprynskyy, B. Buszewski, Colloids Surf. B (2014) 120, 21–27

B. Buszewski, K. Rafińska, P. Pomastowski, J. Walczak, A. Rogowska, Colloids Surf. A (2016) 506, 170–178

A. Król, P. Pomastowski, K. Rafińska, V. Railean-Plugaru, B. Buszewski, Adv. Colloid Interface Sci. (2017) doi: 10.1016/j.cis.2017.07.033

P. Pomastowski, M. Sprynskyy, P. Žuvela, K. Rafińska, M. Milanowski, J.J. Liu, B. Buszewski, J. Am. Chem. Soc. (2016) 138, 7899-7909

V. Railean-Plugaru, P. Pomastowski, M. Wypij, M. Szultka-Młyńska, K. Rafińska, P. Golińska,

H. Dahm, B. Buszewski, Journal of Applied Microbiology (2016) 120, 1250–1263
B.Buszewski, V. Railean-Plugaru, P. Pomastowski, K. Rafińska, M. Szultka-Mlynska, P. Golinska, H. Dahm, J. Microbiol. Immunol. Infect. (2016)

Required initial knowledge and skills of the PhD candidate

- ➔ Analytical thinking
- ➔ Eager to learn
- ➔ Eager to work hard
- ➔ Understanding of colloidal chemistry and theory of adsorption
- ➔ Knowledge about microbiology
- ➔ Knowledge about advanced instrumental techniques: gel electrophoresis, FT-IR, Raman, Light microscopy, electron microscopy (TEM, SEM), mass spectrometry especially MALDI-TOF/TOF MS techniques, chromatography hyphenated with mass spectrometry especially HPLC-ESI-MS

Zgłaszającyprojekt/ Author of the project	
prof. dr hab. Bogusław Buszewski czł. koresp PAN, dr h.c. mult stopień/tytuł, imię, nazwisko	bbusz@umk.pl e-mail
	Wydział Chemii UMK, Katedra Chemii Środowiska i Bioanalityki, jednostka organizacyjna
Proponowani promotorzy i mentorzy/prospective supervisors	
1) promotor główny/ main supervisior	
prof. dr hab. Bogusław Buszewski czł. koresp PAN, dr h.c. mult stopień/tytuł, imię, nazwisko	bbusz@umk.pl e-mail
	Wydział Chemii UMK, Katedra Chemii Środowiska i Bioanalityki jednostka organizacyjna
2) promotor pomocniczy / co-supervisor	
dr Paweł Pomastowski	