

tory activity produced by TRSase Ap₃A, is to stimulate the synthesis of NO and start a cascade of regulatory anti-inflammatory and antioxidant responses.

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Dinucleoside polyphosphates as key regulators of biological processes in tissues and body: molecular aspects of their synthesis by aminoacyl-tRNA synthetases

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Dinucleoside polyphosphates (Ap_nA) have a large range of regulatory functions at different levels of organisms, ranging from the highest forms of multicellular till lower unicellular forms. The importance and universality of regulatory functions of the Ap_nA follows from the known role of this metabolite in the formation of microbial biofilms in the implementation of adaptive features named 'quorum sensing' in communities of microorganisms. Acting as a second messenger as extracellular signaling molecules, Ap_nA can function as neurotransmitter regulators of blood flow as a vasoconstrictor and vasodilator, depending on the length of the phosphate chain. Ap₃A, Ap₄A activate or inhibit the development and the ability to aggregate platelets, stimulate the biosynthesis of DNA in cells. Based on our findings and the literature data, is well established that one of the main sources of the Ap_nA in the body of higher organisms are aminoacyl-tRNA synthetases (ARSase), in particular, tryptophanyl-tRNA synthetase (TRSase). TRSase is involved in the synthesis of Ap₃A, but not Ap₄A, due to the presence of discovered by us significant for catalytic activity zinc ion in the enzyme. The presence of a wide range of regulatory functions of TRSase which is synthesized primarily as apoenzyme, requires specific, well-functioning regulatory mechanisms at the level of protein that can switch from the basic canonical to additional, non-canonical activity. High importance of the non-canonical regulatory activity of the Ap₃A synthesised by TRSase confirms the involvement of these metabolites in the startup stages of defense reactions in the body, in particular the synthesis of nitrous oxide (NO) in the tissue, which is one of the key regulators of homeostasis of the cardiovascular system.

The studies revealed a subtle mechanism of interactions of different types of covalent modification of the TRSase with substrates and intermediates of enzymatic reactions (Trp, PPI, ATP) and, in particular, with zinc for tissue-specific implementation of the non-canonical functions, including the controlled synthesis of an important secondary messenger of cells and tissues – Ap₃A.

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Thermal stability and energy of deactivation of immobilized cell wall invertase in natural and synthetic hydrogel polymers

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The greatest amount of invertase in the cell of yeast *Saccharomyces cerevisiae* is located in the cell wall. Enzyme immobilization offers technical and economic advantages. Industrial applications of immobilized biocatalysts have been gaining importance in

recent decades as they are widely used. Hydrogel materials are increasingly studied for application in biological sensing, drug delivery, tissues regeneration and food industry for number of reasons.

The aim of this work was comparison some of important kinetic parameters for enzyme reaction of a cell wall invertase immobilized in a few hydrogel carriers. Enzyme was immobilized in different natural (alginate and gelatin) and synthetic polymers (polyacrylamide). Concentration of enzyme in all immobilized biocatalyst was the same. Differences in activity of obtained biocatalyst were observed, while no significant difference in pH and temperature optima, as well as in activation energy and Michaelis-Menten kinetics parameters were shown. Significant difference between these biocatalysts was observed in their thermal stability. Thermal stability of free and immobilized cell wall invertase was determined by monitoring the enzyme activity at a few different temperatures between 50 and 70°C. Deactivation constants (k_d) were calculated from Arrhenius equation for all biocatalysts (free and immobilized) for various working temperatures. Obtained k_d values are plotted in the form of Arrhenius plot, that is \ln of k_d against the inverse of absolute temperature, yielding the energy of deactivation, as the angular coefficient of the adjusted straight line, times the universal gas constant. The obtained values are 326, 592, 620 and 706 kJ/mol for free, polyacrylamide, alginate and gelatin immobilized cell wall invertase, respectively. Therefore, increase of the energy of deactivation of immobilized enzyme shown greater stability than free enzyme, but the greatest stability was proven for enzyme immobilized in gelatin hydrogel.

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Immobilization of NAD⁺/NADH on magnetic nanoparticles and its selective oxidation and reduction reactions with mediated by galactitol- and lactate- dehydrogenases

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Redox reactions in biochemistry have an important function and they are catalyzed by dehydrogenases and oxidoreductases. These reactions have large application by bio fuels, sensing technology and biotransformation for the production of several interesting materials.

The oxidoreductases are working with co-factors NAD⁺/NADH and their recycling are essential for economical reason. The recycling and reusability is a big challenge in bio fuel, sensing and biotransformation technologies. Without recycling the use of oxidoreductases are more expensive than their products. Many attempts are made for the reusability these materials. The solution of this problem is in the same time the solution associated with hydride (hydrogen) generation. This study focus on the synthesis of NAD⁺/NADH functionalized Fe₃O₄ magnetic nanoparticles and their application on the redox enzyme reactions for developing a method which enable ease regeneration and reuse of cofactors.

First, silica coated magnetite particles were synthesized and 3-Aminophenylboronic acid (APBA) was attached on the surface of particles. Nicotinamide adenine dinucleotide (NAD) was immobilized on the magnetically responsive APBA attached magnetic support. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and transmission electron micrograph (TEM) methods were used to characterize the surface modified magnetic nanoparticles.