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Regulation of Biocatalysis with the α-Chymotrypsin–Bowman–Birk Inhibitor Pair Immobilized on Magnetite-Gold Hybrid Nanoparticles Using a Non-Heating Low-Frequency Magnetic Field

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Abstract—Magnetite-gold hybrid magnetic nanoparticles of the dumbbell type were used to immobilize two proteins: α-chymotrypsin and the Bowman–Birk inhibitor. It is shown that under the influence of a low-frequency magnetic field (50 Hz), the activity of chymotrypsin bound in a complex with an inhibitor is drastically reduced depending on the magnetic field induction. We hypothesize that this effect is related to the aggregation of magnetic nanoparticles under the influence of a magnetic field.

Keywords: magnetic nanoparticles, enzyme immobilization, low-frequency magnetic field, nanoparticle aggregation, biocatalysis regulation

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Magnetic nanoparticles (MNPs) are extremely interesting as carriers for the immobilization of enzymes. They have a large specific surface, easily transfer the substrate's mass to the surface (which is characteristic of most nanosized carriers for the immobilization of enzymes), and in addition, they respond to an external magnetic field. Due to these properties, they can be used to isolate enzymes from the culture medium [1], create biosensors [2], and control biocatalysis processes [3–5]. The magnetic nanoparticles transform the energy of an external alternating magnetic field (AMF) into physical or chemical changes that occur in biomolecular structures interacting with them. These changes can be activated by magnetic hyperthermia [3, 6] and vibrational rotational motions of MNPs [7–9], as well as by processes of their aggregation and disaggregation [4, 10]. Magnetic hyperthermia based on the transformation of the high-frequency magnetic field energy (f = 200–600 kHz) into thermal energy due to the relaxation of the magnetic moments of MNPs in AMF usually leads to the partial or complete inactivation of enzymes due to their extremely low temperature stability. Using the magneto-nanomechanical approach based on bringing MNPs into the vibrational-rotational motion under the action of AMF, it was possible to increase the activity of chymotrypsin bound in a complex with a trypsin inhibitor [11]. However, as examples of controlling biocatalytic processes in the presence of MNPs, the studies in which the magnetic field leads to the MNPs' aggregation or disaggregation, resulting in an increase in the rate of the enzymatic reaction, are the most interesting ones. Thus, it has been shown that the magnetic-field-stimulated aggregation of two MNP populations, on the surface of one of which an enzyme was immobilized, and on the surface of the other, a substrate, leads to their colocalization and activation of the enzymatic reaction [4]. It has been demonstrated that in a rotating magnetic field, the activity of lipase immobilized on MNP aggregates increases, depending on its frequency [10]. The authors attribute this effect to the disaggregation of nanoparticles, as a result of which the active site of

Abbreviations: MNPs, magnetic nanoparticles; AMF, alternating magnetic field; LF, low-frequency; 4-N-DOPA, 4-nitrodopamine; EDC, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride; S-NHS, 4-sulfo-*N*-hydroxysuccinimide; CT, α-chymotrypsin; BBI, Bowman–Birk inhibitor; NSAAPFpNA, *N*-succinyl-alanyl-alanyl-prolyl-phenylalanyl para-nitroanilide; PDAM, 1-pyrenyldiazomethane; PEG, polyethylene glycol; NTA, nanoparticle trajectory analysis; TEM, transmission electron microscopy.