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Dephosphorylation of lipopolysaccharides by alkaline phosphatase from marine bacterium

Key words: marine bacterium, alkaline phosphatase, lipopolysaccharide, dephosphorylation

The recombinant alkaline phosphatase from the marine bacterium *Cobetia amphilecti* KMM 269 (CmAP) with a higher activity (≥ 12,000 U / mg) among the known analogues was previously biochemically characterized [1]. However, the biological role of the extracellular highly active alkaline phosphatase (AP) of the marine bacterium remains unknown. In addition, the *C. amphilecti* KMM 296 genome has been found the presence of several genes encoding alkaline phosphatases, probably associated with still unexplored functions for the marine life style [1]. The recent discovery of new properties and biological functions of AP from various sources shows the prospect of using them as drugs for various purposes of medicine and biotechnology [1, 2]. Thus, intestinal APs was able to dephosphorylate lipopolysaccharides (LPS, endotoxins) of bacteria that resulted in a decrease of the overall inflammatory process [2]. It is known that LPS are major components of the cell envelope of gram-negative bacteria, which are an important contributing factor to septic shock, in general, and gram-negative septic shock, in particular. The endotoxic properties of LPS depend on the structural features of lipid A, a phosphoglycolipid. Currently, gram-negative sepsis and endotoxic shock are a serious clinical problem. They give a high percentage of deaths even in countries with a developed health system. Modern medicine does not have specific and effective drugs for anti-endotoxin therapy. Currently, gram-negative sepsis and endotoxic shock are a serious clinical problem. They give a high percentage of deaths even in countries with a developed health system. Modern medicine does not have specific and effective drugs for anti-endotoxin therapy.

One of the approaches used to reduce the toxicity of LPS is dephosphorylation of lipid A. The cleavage of a single phosphate group in lipid A by mild acid hydrolysis of LPS has been shown to cause a significant weakening of its pyrogenicity and toxicity [3]. In this work, we first studied the effect of *Cm*AP on the lipopolysaccharides *Escherichia coli* S-LPS-055: B5 and Ra-LPS-EH100 (Sigma). The total content of phosphorus in the initial solutions of LPS and after their treatment with AP followed by dialysis against water to remove free phosphate groups was determined by the method described [4]. It was found, that *Cm*AP exhibits enzymatic activity against LPS, which largely depends on the aggregate state of the LPS molecules. The greatest activity of the

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enzyme removing up to 90% phosphorus was observed with LPS in the monomeric form. For complete dissolution of LPS, parameters such as sample concentration, buffer composition and pH, as well as the incubation temperature, were selected.

Thus, new data on the dephosphorylating activity of *Cm*AP against LPS are a promising basis for developing a new therapeutic approach with the use of alkaline phosphatase for neutralizing the effects of bacterial endotoxins (sepsis, endotoxic shock).

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