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USE OF SUMO-EXPRESSION SYSTEM AND SUMO-PROTEASE FOR PRODUCTION OF ACTIVE INTERFERON α-2B

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Regular problems of recombinant proteins expression and production include incorrect protein conformation, toxicity to the host cell and low protein yield. These issues are usually resolved by using an alternative host or expression systems and fusing the protein of interest. Among a wide variety of expression systems, the protein SUMO-fusion (Small Ubiquitin Like Modifier) system with a SUMO-tag is characterized by ease, efficiency and precision with which the SUMO-tag is removed by SUMO-protease [1]. Attachment of SUMO to the N-terminus of a protein of interest can improve protein solubility, achieve correct protein folding, N-terminal formylmethionine elimination and increase total yield by enhancing expression and decreasing degradation [2, 3]. High specificity of the enzyme action, simplified stages of isolation, purification and identification of the target protein are the key advantages of this system [1–3].

Aim. The aim of the study was to obtain SUMO-protease and check the functional activity of the enzyme for further use in the production of active pharmaceutical ingredients, for example, for interferon α-2b manufacture.

Methods. Competent cells *E. coli* BL 21 from the established research bank were used for the experiments. They were transformed with plasmid DNAs with the target genes SUMO_protease and SUMO_IFN, based on the pET-24 expression vector provided by the laboratory. Transformation was performed according to the protocol using heat shock [4]. After clones screening, inoculum obtaining and preparations, fermentation was performed. Protein expression was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). SUMO-protease was purified by immobilized metal ion affinity chromatography (IMAC). The isolated SUMO-interferon inclusion bodies were solubilized and refolded. After that, reaction between the obtained enzyme and substrate studied. The proteolysis intermediate and final products were analyzed by SDS PAGE and RP-HPLC.

Results and Discussion. The results confirm functional activity of the obtained SUMO-protease enzyme and demonstrate high specificity to SUMO-interferon fusion protein. At the same time, the obtained SUMO-interferon solution after refolding contains mainly the target protein in a correct conformation and a small amount of impurities, compared to the refolding products of interferon α-2b, which does not contain SUMO-tag. The refolding solution can be used directly for enzyme cleavage using SUMO-protease without additional purification steps.

After enzyme was added to the substrate (the sample was taken 1 hour after-enzymolysis start (Figure, *A*), fusion SUMO-interferon was cleaved into SUMO-tag and mature interferon α-2b. When the next sample was analyzed (3 hours after enzymolysis started (Figure, *B*), a considerable decrease in the peak area, corresponding to fusion precursor, and a parallel increase in the peak area corresponding to interferon α-2b were observed. According to the results, the interferon α-2b yield exceeds 80%.

Conclusions. SUMO-protease was obtained and functional activity of the enzyme was tested for further use in active pharmaceutical ingredients production. The enzyme possesses a highly specific activity in SUMO-tag cleavage. The relative purity and increased overall yield of the

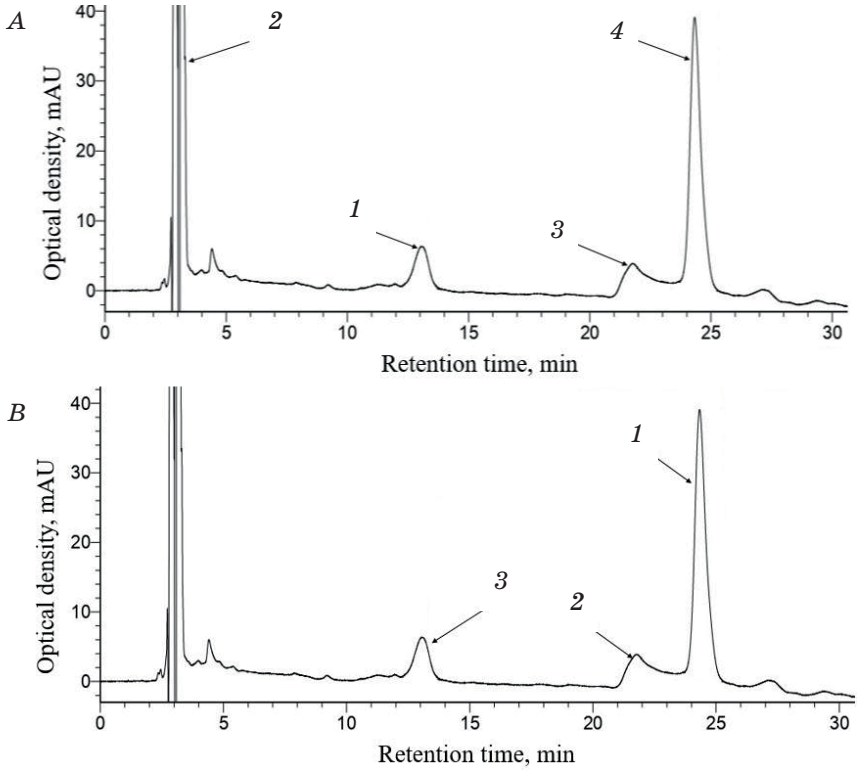

Fig. **Chromatographic profile of the proteolytic reaction products**
A — 1 hour after enzymolysis start; *B* — 3 hours after enzymolysis start;
1 — SUMO-interferon; *2* — unbound fraction (contains a SUMO-tag); *3* — contaminants;
4 — interferon α-2b

product suggests that the use of SUMO-expression system can be considered as a good choice for pharmaceutical recombinant proteins manufacturing technology, which facilitates not only N-terminal formylmethionine elimination, but also yield improvement and further purification cost reduction.

Key words: SUMO-protease, SUMO-interferon, SUMO-tag, interferon α-2b.

Authors' contribution. I-MMK worked with cell culture and proteins extraction, carrying out proteolysis and SDS PAGE, MMS is responsible for analysis of the results, editing, and supervision.

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