Characterization of the protease with keratinolytic activity produced by Aspergillus giganteus

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S. Timorshina[I], A. Osmolovskiy[I]

[I]Lomonosov Moscow State University, Moscow, Russia

Proteases are in demand in various fields of science and bioeconomy. In recent years, a substantial part of studies has been devoted to investigation of keratinases. Expanding the accumulated knowledge about biochemical and physicochemical properties of keratinolytic enzymes, will increase the efficiency of processes that are significant for humanity, such as biodegradation, treatment of certain skin diseases, and production of bioplastics from organic waste. In this regard, this work was focused on study of the keratinolytic protease synthesized by Aspergillus giganteus. A complex preparation of proteins secreted by A. giganteus was fractionated by isoelectric focusing. Enzymatic activity was examined with keratin suspension. Also, reactions were carried out with various chromogenic peptide substrates para-nitroanilides (CPS) to determine the substrate specificity of the enzyme. For inhibitory analysis, the enzyme was treated (1 h, 25 °C, pH 8.2) with the following inhibitors: PMSF (1.5 mM), EDTA (1 mM), PCMB (1 mM), TPCK (0.5 mM), TLCK (0.5 mM), soybean trypsin inhibitor (0.5 mg/ml). Reaction conditions are 37 ° C, pH 8.2, 600 rpm. The amount of formed products in all types of reactions was measured spectrophotometrically. The purified enzyme was analyzed by SDS-PAGE. Qualitative reaction was performed for determination of glycoproteins using periodic acid and Schiff's reagent by dot blotting on nitrocellulose membranes. The keratinolytic enzyme of A. giganteus is a protein with molecular weight about 27 kDa and pI 9.2-9.3. The highest activity in reactions with CPS was shown with Z-Ala-Ala-Leu-pNA, and protease inhibition occurred upon the addition of PMSF and EDTA. These results may indicate the subtilisin-like nature of the enzyme. No glycosylation of the enzyme was detected, which will simplify the cloning work. Thus, some characteristics of new keratinolytic protease that can be promising for practical applying and produced by A. giganteus were investigated.