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Hydrolysis of native proteins by keratinolytic protease of *Doratomyces microsporus*

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Abstract

A keratinolytic protease from the fungus *Doratomyces microsporus* was investigated for its ability to hydrolyse different native proteins. The purified enzyme was incubated for up to 24 h with keratinous substrates as well as with non-keratinous proteins. The results showed that the enzyme was broad specific since it hydrolysed various globular and fibrillar proteins. The hydrolysis of keratinous substrates decreased in the following order: skin keratins > nail keratins > hair keratins. With non-keratinous substrates, the order was: casein > BSA > elastin. Feather keratin and collagen could not be hydrolysed. Comparison of the enzyme with some known proteolytic enzymes showed that on keratin from *stratum corneum* the activity of the keratinase was comparable to that of proteinase K, other enzymes were less active. Hydrolysis of porcine skin with the keratinase revealed the degradation of the epidermis while dermis was not damaged.

Keywords: Hydrolysis; Keratinolytic protease; *Doratomyces microsporus*

1. Introduction

Keratins, the structural proteins in vertebrates, are the main constituents of skin and its appendages and are also found in other epithelial tissues within the body. They are the most abundant members of the superfamily of intermediate filaments. Their specific feature is a high content of sulphur and high resistance to degradation.

In nature, keratins are hydrolysed by some microorganisms which synthesise keratinolytic enzymes. The best known among them are pathogenic fungi causing dermatophytosis or candidosis, and bacteria used for keratin waste degradation. The keratinases are mainly extracellular serin type proteases, except for yeast enzymes which are aspartic proteases. The molecular masses of the enzymes range from 20 to 50 kDa and most of them are optimally active at temperatures up to 50 °C, but also some thermostable keratinases have been found with the activity up to 90 °C. The enzymes belong to the most active proteinases and are broadly specific. Their potential application is in different fields where keratins should be hydrolysed, such as medicine, cosmetics, detergent and leather industry or waste bioconversion, but also for obtaining specific amino acids and peptides from proteins or in biotransformation using proteolytic enzymes.

Primary screening of about 300 non-pathogenic fungi was performed to find potent keratinase producers [1]. The selected strains were cultivated in a submerged aerobic fermentation in a medium optimised for the keratinase synthesis. Among the most active was a strain of the hyphomycete *Doratomyces microsporus*. Its extracellular keratinase was isolated and purified by chromatographic methods. The main

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biochemical characteristics important for the application of the enzyme were determined [2] and the conditions for the release of soluble proteins from keratins by the enzyme were optimised [3]. It was found that the keratinase of *D. microsporus* belongs to serin proteinases, which is most active at slight alkalic environment at increased temperature. To elucidate its substrate specificity, experiments were performed for a more detailed study of its capability to hydrolyse different proteins.

2. Experimental

For this investigation, substrates were prepared by grinding or cutting and defatting of several keratinous materials of different origin, such as *stratum corneum* from human sole, human and porcine nail, human hair and chicken feathers. Bovine skin keratin and sheep wool were purchased by commercial suppliers. As non-keratinous substrates, two fibrillar proteins from skin, collagen and elastin were chosen while globular proteins were represented by bovine serum albumin (BSA) and casein. Keratinase purified by hydrophobic interaction- and gel chromatography was used in the experiments of hydrolysis of the selected substrates. The increase of soluble products after incubation of enzyme/substrate mixture at 37 °C for a given time period was a criterium for hydrolysis amount. For comparison of the keratinase with the known proteinases, the following enzymes were purchased by commercial suppliers: proteinase K, elastase, collagenase, subtilisin, trypsin and chymotrypsin. All enzymes were tested under the same experimental conditions and compared on the basis of their specific activity.

3. Results

Hydrolysis of different keratinous substrates during 30 min incubation with the keratinase showed that *stratum corneum* keratin from human sole was the preferred substrate. Relative specific activity of the enzyme decreased in the following order: *stratum corneum* 100%, bovine skin keratin 38%, human nail 19%, porcine nail 8%. With human hair, sheep wool or chicken feathers, no degradation products could be detected after 30 min incubation at 37 °C. The time course of hydrolysis of *stratum corneum* was compared with that of the non-keratinous substrates. It was shown that the two fibrillar proteins, elastin and collagen, were only slightly affected by the enzyme, while the degradation of BSA and especially of casein was much faster and gave greater amount of soluble products. To investigate whether for hydrolysis of some more resistant substrates the enzyme needed more time, the incubation was prolonged to 24 h. The results confirmed that chicken feather cannot be hydrolysed by the keratinase of *D. microsporus* but it was shown that human hair was hydrolysed in a prolonged action. The amounts of soluble products produced during enzyme reaction decreased in the following order: human *stratum corneum* > bovine skin keratin > human nail > human hair > porcine nail. With non-keratinous substrates, the preferred substrates were in the order: casein > BSA > elastin. When the results were compared with the hydrolysis of *stratum corneum*, it was shown that it is only slightly more resistant to enzymatic attack than BSA. On the other hand, collagen could not be hydrolysed even after 24 h incubation.

To evaluate the activity of the new keratinase in relation to some known proteolytic enzymes, experiments were performed with some commonly used commercial proteases of fungal, bacterial or animal origin. The activity of the enzymes was measured on human *stratum corneum* keratin and on casein. The results showed that on keratin, proteinase K was most active, however, the keratinase of *D. microsporus* gave only slightly lower yield of soluble products but it increased with the same velocity. Much less active were subtilisin, elastase and trypsin, followed by chymotrypsin. Collagenase was not active at the given experimental conditions. When casein was hydrolysed keratinase was slightly more active than proteinase K. Both enzymes were closely followed by the third microbial enzyme, subtilisin. Less activity was shown by elastase, followed by chymotrypsin and trypsin. Collagenase could not hydrolyse casein.

From the results of hydrolysis of different proteins by the keratinase of *D. microsporus*, it was concluded that the enzyme could be suitable for the application in leather industry, since it is able to degrade *stratum corneum* keratin and elastin but not collagen which is the main constituent of the dermis. Therefore, the experiments were performed to evaluate the effect of

the enzyme on crude porcine skin. The cuttings of skin were soaked in enzyme solutions and the effect on tissue was followed by microscopic examination of thin sections. It was found that the lower layers of the epidermis were disintegrated. Apparently *stratum corneum* was not much affected but was detached from the lower layers of the epidermis as a whole. The dermis was not degraded by the enzyme. The observation of the cross-section of the root sheaths of porcine hair revealed that the outer root sheath was disintegrated. According to the literature [4], most probably the keratin pair K5/K14 is the substrate for the keratinase of *D. microsporus*.

4. Conclusions

The keratinase of the fungus *D. microsporus* is broadly specific, it degrades keratins and non-keratinous proteins. Except for chicken feather being resistant to enzyme action, all keratinous substrates are hydrolysed but by a different degree. Human skin keratins are most affected, hair is least hydrolysed. Among non-keratinous substrates, fibrillar and globular proteins are substrates for the enzyme, with exception of collagen and gelatin. The keratinase is comparable to a commercial enzyme proteinase K. Its ability to disintegrate epidermis while leaving dermis intact suggests that the enzyme is suitable for the application in leather industry.

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