Human Skin Proteases.
Fractionation and Characterization

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Summary. Proteolytic enzymes of human skin extracts were fractionated by using Sephadex G-100 and Sepharose 6 B gel filtrations. The preparations obtained were characterized enzymologically. Several fractions containing proteases with clearcut enzymatic characteristics were obtained.

Hydrolysis of ATEE (acetyl tyrosine ethyl ester), casein, BAPA (benzoyl arginine p-nitroanilide), BAEE (benzoyl arginine ethyl ester) and TAME (tosyl arginine methyl ester) was found to take place by the first enzyme preparation. The pH-optima were at 7.8—8.2. BAPA, BAEE and TAME were hydrolyzed by a trypsin-like protease that is different from the protease(s) hydrolysing ATEE and casein. These proteases showed a very high molecular size, estimated on the basis of elution in Sepharose 6 B gel, but were not definitely separated from each other. Separate enzymes in the first preparation hydrolyzed BANA (benzoyl arginine naphthylamide) optimally at pH 5.8 and TEE (tyrosine ethyl ester) optimally at pH 7.7.

The second protease preparation hydrolyzed BAEE optimally at pH 8.2 and TEE optimally at pH 7.7. Two separate proteases were present in this preparation.

The third protease preparation hydrolyzed casein optimally at pH 5.8 and hemoglobin optimally at a lower pH. The enzymes were considered to belong to cathepsin D and E group of proteases.

The fourth preparation hydrolyzed BANA (and also BAEE) optimally at pH 5.8; the reaction was affected by SH-activators and chelating agents; the enzyme thus resembles cathepsin B1 (B').

The fifth preparation hydrolyzed TEE with pH-optima at pH 6.8 and 8.0. The reactions at these pH-values showed different characteristics and may thus be due to two separate esteropeptidases of a low molecular size.

Einzelne Enzyme der ersten Präparation hydrolysierten BANA (Benzoylnaphthylamid) bei einem pH-Optimum von pH 8, und TEE (Tyrosinäthylester) bei pH 7,7.


Die fünfte Fraktion hydrolysierte TEE mit einem pH-Optimum von 6,8 und 8,0. Bei diesen pH-Werten zeigten die Reaktionen unterschiedliche Charakteristika, die möglicherweise auf das Vorliegen von 2 verschiedenen Esteropeptidasen mit einem niedrigen Molekulargewicht zurückzuführen sind.

Introduction

The pattern of proteolytic enzymes of human skin has been found very complex and much work remains to be done until all proteases have been separated and enzymatically characterized [20]. The human skin protease pattern resembles closely that of the rat skin although some definite differences are known [11, 20].

The differential extraction of proteases and of a protease inhibitor of the human skin has been recently studied by us [4]. The proteases and their inhibitor(s) were easily separated from each other by using stepwise extraction in buffers containing different salt concentrations. The inhibitor(s) and some of the enzymes, especially those with acidic pH-optima, were extracted abundantly in a dilute buffer while some others were extracted only in a buffer containing high salt concentration, in KSCN or in butanol [4].

In this paper we want to report our findings concerning the partial chromatographic separation of human skin proteases and characterization of the preparations obtained. The substrates used in enzyme assays were those generally used in studies dealing with skin proteases, i.e. casein, hemoglobin, BANA (benzoyl arginine naphthylamide), BAPA (benzoyl arginine p-nitroanilide), BAEE (benzoyl arginine ethyl ester), TAME (tosyl arginine methyl ester), ATEE (acetyl tyrosine ethyl ester), TEE (tyrosine ethyl ester) [10]. This kind of study serves as a basis for further studies on the identity, characteristics and origin of the proteases present in human skin and sweat [5].

Material and Methods

Skin Samples and Extraction Procedures

Skin samples were obtained and prepared as earlier [4]. The minced skin was homogenized with Ultra-Turrax homogenizer for 1 min and extracted in ten volumes (w/v) of 10 mmol/l phosphate buffer, pH 8.0 (buffer extract = B-extract). The