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ELASTOLYTIC ENZYMES IN SURFACE WASHINGS OF HUMAN SKIN

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Abstract. The hypothesis that the enzymes of the elastase complex play a role in the metabolism of elastin and in maintenance and/or degeneration of the skin elastic tissue depends on the assumption that these pancreatic enzymes pass into the skin via the circulation. Since the presence of these enzymes can be demonstrated in blood and in the vessel wall, experiments were started to investigate if they could be detected in human skin. The results of the study clearly show that small amounts of elastoproteinase, elastomucase and the elastoproteinase inhibitor are sometimes present in the washings of the human arm.

Balo (1) has pointed out that the elastolytic enzymes of the elastase complex may be decisive in the decomposition of elastic fibers as well as in their synthesis and, moreover, that they are essential for the maintenance of the intact elastic tissue as a whole. Most of these investigations have been carried out with ligamental elastin, aortic elastin and lung tissue, but little attention has been paid to the skin elastic tissue. The susceptibility of skin elastin to elastase digestion and changes in the concentration of the serum elastoproteinase inhibitor have been studied in pseudoxanthema elasticum, senile and solar elastosis, nevus elasticus, striae distensae, lax skin, Ehlers-Danlos Syndrome and some other dermal diseases (2, 5–7, 10–11, 16, 22, 23).

Until recently, the enzymes of the elastase complex could only be isolated and purified from pancreatic powder and were found in the pancreatic juice and in urine. In recent years, however, the presence of these enzymes and enzyme inhibitors were demonstrated in the circulation, in some tissue cultures and in bovine aortic wall (13). The presence of collagenase in rat skin (9, 18) and collagenolytic activity in tissue culture specimens of human skin (3, 19) also prompted investigations into elastolytic enzymes in human skin.

MATERIAL AND METHODS

In an investigation of the effect of alkaline soaps on the skin it was found that concentrated washings of the arms of volunteers sometimes contained small amounts of proteins. One of these proteins could be located in the paper electrophoretogram in exactly the same place as the proteolytic elastase component. This technique has been used in our experiments.

The hand and arm of a volunteer was washed thoroughly with soap and water and then soaked for 30 min in a 2 l graduated glass cylinder filled with a Teorell-Stenhagen buffer of pH 11, heated to 37°C. Hand and arm were frequently rotated in the cylinder. The temperature of the buffer decreased during this period to about 30°C. The skin extract was then neutralized to pH 6–7, rapidly cooled to 4°C, filtered on a Buchner and concentrated in a rotating vacuo-evaporator (Rotavapor, Buchli, Switzerland). The concentrated extract was dialyzed against distilled water in the cold room for three days with several changes of the water and finally lyophilized. In some cases a precipitate was formed during dialysis. This precipitate was collected and dried with acetone. Care was taken that the arm and hand of the volunteer did not show any healing wounds or other damaged area.

The buffer used consisted of 0.004 *M* citric acid, 0.006 *M* phosphoric acid, 0.006 *M* boric acid, 0.029 *N* sodium hydroxide and a sufficient amount of hydrochloric acid to adjust the pH of the buffer to 11.

The dried extracts were investigated for enzyme and inhibitor activity on alkali-treated elastin at pH 8.7 and on acid-treated elastin at pH 7.2. These two substrates are routinely used in our laboratory for the determination of the activity of elastoproteinase, elastomucase (the mucolytic elastase component), and the elastoproteinase inhibitor (12). The enzyme system for measuring elastoproteinase activity in skin extracts consisted of 50 mg elastin homogenized in 10 ml of a borate buffer ($\Gamma/2 =$

0.135; pH 8.7 or 7.2) and x mg dried extract. For the determination of the activity of elastomucase and elastoproteinase inhibitor, 0.05 or 0.1 mg of a highly purified elastoproteinase preparation was also added to the enzyme system (the presence of elastomucase in the skin extract enhances the activity of elastoproteinase; the concentration of the inhibitor can be calculated from the reduced enzyme activity). The enzyme system was incubated for 3 hours at 37°C. The amount of elastin solubilized was measured by the biuret method (12).

The elastoproteinase activity is expressed in enzyme units as follows: E.U. = amount of enzyme solubilizing 50% of the total amount of elastin in the reaction mixture under the experimental conditions used (E.U./mg = elastoproteinase units per mg dried skin washing).

The elastomucase activity is expressed in Em.U. (amount of elastomucase enhancing the activity of 0.05–0.1 mg of a highly purified elastoproteinase by 50% under the experimental conditions used; Em.U./mg = elastomucase units per mg dried extract).

The elastoproteinase inhibitor activity is expressed in I.U. (amount of inhibitor reducing the activity of 0.1 mg of highly purified elastoproteinase by 50% under the standard conditions; I.U./mg = inhibitor units per mg dried skin washing).

One of the disadvantages of heterogenous enzyme systems is the fact that the enzyme reactions depend largely on the particle size of the substrate and subsequently on the stability of the elastin suspension between inversions of the reaction tubes. The accuracy of the measurements of the various enzyme activities has been studied in a large number of experiments. The standard deviation calculated from triplicate determinations of the activity of elastoproteinase is about 5% and that of the activity of elastomucase about 10%. Under the experimental conditions used in the experiments with skin extracts an elastoproteinase activity of 0.02 E.U./mg, an elastomucase activity of 0.05 Em.U./mg and an elastoproteinase inhibitor activity of 0.04 I.U./mg can be detected reliably.

From most of the skin washings paper electrophoretograms were performed with the Beckman paper electrophoresis apparatus. (Djurrum type; Veronal buffer of $\Gamma/2 = 0.075$ and pH 8.6; Whatman 3MM paper strips: constant current of 14 mA; mean voltage of 160 V; running period 11 hours). The paper strips were dried at 120°C and stained with Amido Black 10 B. In this way the bands of 60 μg of enzyme give a just-visible staining.

RESULTS

Table I and Fig. 1 are examples of the results obtained with this technique. The yield of washings varied widely. Sometimes a large amount of material completely insoluble in veronal or borate buffers was present (see the dark colored protein band on the line of application in the paper electrophoretograms). In some cases only this electrophoretically immobile material could be detected

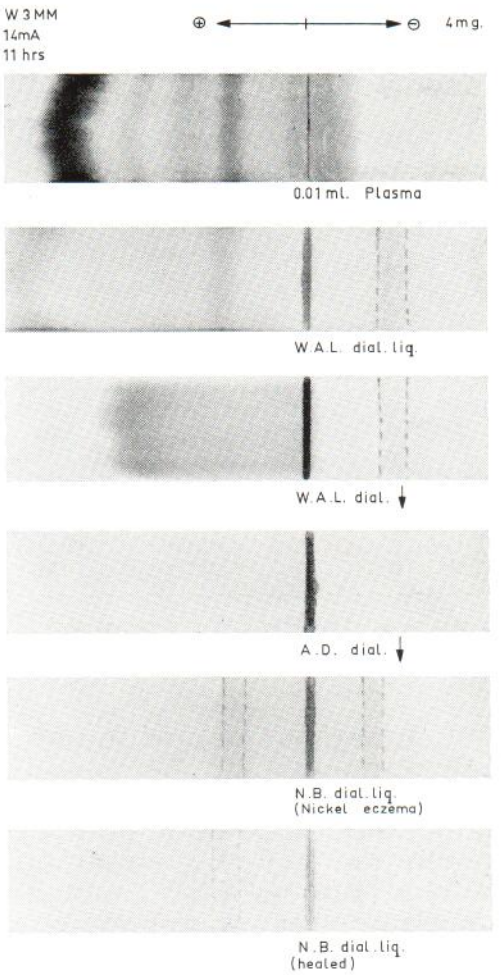

Fig. 1. Paper electrophoretograms of alkaline skin extracts of human arms, sometimes showing the protein band of the enzyme elastoproteinase (band between the dotted lines at the cathodic side of the line of application). For technical data see text.

in the skin washings. In other cases the elastoproteinase band on the cathodic side of the paper strip and some kinds of β- and/or α_2-globulin-like proteins on the anodic side were present (as compared with the paper electrophoretogram of human serum run under the same experimental conditions).

Table I shows that in the skin washings of some volunteers, measurable amounts of elastoproteinase could be detected in spite of the large amount of inert protein material. This material influenced the exact weight of the soluble protein (enzyme activity expressed in units per mg of

Table I. *Elastolytic activities in washings of the human arm (Teorell–Stenhagen buffer of pH 11)*

Name	Sex	Age	Yield	Alkali-treated elastin pH 8.7			Acid-treated elastin pH 7.2		
				E.U./mg	Em.U./mg	I.U./mg	E.U./mg	Em.U./mg	I.U./mg
C. P. O.	♀	21	52 mg	0.04	0.02	—	0.03	0.09	—
A. D.	♀	?	19 mg	0.09	0.10	—	0.07	0.08	—
P. L.	♀	33	160 mg	0.01	—	0.14	0.01	—	0.49
W. A. L.	♂	41	186 mg	0.39	0.04	—	0.29	—	0.04
			dial. ↓						
			17 mg	0.41	0.05	—			
B. J.	♂	43	194 mg	0.13	0.06	—	0.08	0.03	—
			dial. ↓						
			7 mg	0.03	—	—			
G. V. M.	♂	49	54 mg	0.46	0.07	—	0.34	0.06	—
G. G.	♂	58	207 mg	0.00	—	0.24	0.00	—	0.51
J. L. D.	♂	63	63 mg	0.11	0.05	—	0.03	0.08	—
			dial. ↓						
			8 mg	0.02	0.12	—			
A. S. J.	♀	24	39 mg	0.01	—	0.12	0.02	—	0.21
N. B.[a]	♀	55	97 mg	0.27	0.03	—	0.13	0.41	—
N. B.[b]	♀	55	107 mg	0.01	—	0.32	0.00	—	0.70

[a] Patient with so-called nickel eczema on hand and arm.
[b] Patient after healing of the eczema.

total dried skin washings) but could also intervene in the enzymic reaction.

The elastomucase activity is always extremely small. This is due, in part, to the denaturation of the active enzyme by the alkaline buffer and in part also to the fact that if both enzymes are simultaneously present in such small amounts in the reaction mixture, the elastolytic activity measured in the blank is already the combined effect of elastoproteinase and elastomucase. In other words: what will be measured is the excess of elastomucase. On the other hand, if an excess of enzyme inhibitor is present in the skin washings, no enzyme activity can be measured at all (see Table I: P. L., G. G. and N. B.).

The patient identified as N. B. is noteworthy. After healing of the eczema on her arms, the washings did not contain active enzyme but, on the contrary, showed a high elastoproteinase inhibitor activity. In this connection reference is also made to the data obtained from a patient with a so-called dishydrotic eczema. The paper electrophoretogram of the blister fluid shows the protein band of the elastoproteinase. The fluid itself appears to contain 0.45 EU./ml blister fluid (Fig. 2). In two other cases of this eczema these results could not be reproduced, probably due to the fact that the blister fluid was contaminated with large amounts of serum protein (enzyme activity completely covered by the serum enzyme inhibitor activity).

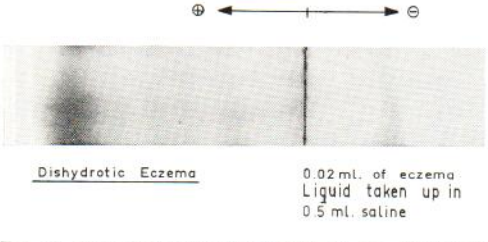

Fig. 2. Paper electrophoretogram of the blister fluid of a dishydrotic eczema diluted with saline. For technical data see text.

DISCUSSION

Elastin is usually held to be an inert protein with a very low metabolic turnover. However, the results of various experiments permit the conclusion that it is more metabolically active than believed hitherto, possibly by reconstitution in situ by binding to acid mucopolysaccharides and other ground substances (21). Subtle molecular events and macromolecular heterogeneity may be responsible for the degenerative reactions in skin connective tissue (4, 25). The concept that the en-

zymes of the elastase complex may to some extent be involved in the maintenance and/or degeneration of skin elastic tissue depends on the assumption that these pancreatic enzymes pass into the skin via the circulation.

The finding of these enzymes in skin washings with alkaline buffers is an indication of their presence in skin itself. Taking into account the high efficiency of the epidermal barrier it is more likely that the enzymes are of epidermal rather than of dermal origin. If the latter is the case, the surface treatment with the pH 11 buffer must have altered drastically the barrier properties of the epidermis. Recently, Yamada & Ofuji (24) demonstrated histochemical evidence of the presence of proteolytic enzymes in human epidermis.

Another explanation of the presence of these enzymes in skin extracts might be their excretion together with sweat secretion. However, the only indication in this direction is the impression that much less material can be extracted from skin with alkaline buffer at 20°C than at 37°C. In any case, the next step will be, identification of elastolytic enzymes in the subepidermal connective tissue.

A bacterial or fungal origin of the elastolytic enzymes can be excluded for the following reasons: (*a*) arms and hands of none of the volunteers showed any inflammatory type of infection (except patient N. B.); no "elastase-producing" dermatophytes or pathogenic fungi were thus present (20); (*b*) the characteristics of two other bacterial elastases (Pseudomonas aeruginosa and Flavobacterium elastolyticum) largely differ from those of pancreatic elastase, e.g. in respect of their place in the paper electrophoretogram, and in regard to pH and temperature optima (14, 15, 17). The fact that the enzyme activities in the skin washings are much less marked than expected from work with pancreatic enzymes is probably not only due to the high pH of the buffer and the temperature during the soaking period of the arm, but can also be explained by the observations of Hall (8) who demonstrated that elastoproteinase exists in two forms: a monomer form (the free enzyme molecule) and the dimer form (two molecules cross-linked by calcium). These two forms of the enzyme act on elastin in a different way and also differ from each other in some other characteristics (e.g. heat lability). Since the Teorell–Stenhagen buffer contains calcium-chelating agents, this may not only have affected the extraction of the enzymes from the skin, but may also inhibit the elastoproteinase activity (in the same way as citric acid and EDTA).

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