N-Terminal Amino Acids Formed during Digestion of Bovine Fibrinogen by Plasmin

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The purpose of this work was to make a qualitative and quantitative estimation of the N-terminal amino acids, formed during the digestion of fibrinogen by plasmin, activated by urokinase. Besides the estimation of the N-terminal amino acids the decomposition of fibrinogen was followed by determination of coagulability. The determinations of N-terminal amino acids were carried out by Edman's phenyl-*iso*thiocyanate method [1]. The coagulability was determined after addition of lysine-ethylester in order to avoid further fibrinolysis [2]. The syneresis method of Morrison, as modified by Saifer and Newhouse [3], was applied.

The fibrinogen was prepared by the method recently published by Blombäck and Blombäck [4] and the coagulability of the preparations used by us varied from 95 to 99 %. Even these very pure preparations contain traces of plasminogen and after activation sufficient amounts of plasmin are formed to digest the fibrinogen.

The urokinase was prepared from human urine by adsorption on Hyflo Super Cel, and elution with ammonia. It was further purified by means of ammonium sulphate fractionation. By this method about 5 mg of dry substance was obtained from one liter of urine and the yield of activity was about 80 %.

500 mg of fibrinogen and 0.5 mg urokinase were dissolved in 50 ml of 0.02 M phosphate buffer pH 7.2 containing NaCl to a concentration of 0.3 M and incubated at 37 °C. At different times 10 ml of the reaction mixture was added to 10 ml pyridine containing 0.5 ml phenyl-*iso*-thiocyanate, thus interrupting the digestion. The coupling was continued for 2 h by keeping pH 8.5—9.0 with occasional addition of 1 N NaOH. The excess of phenyl-*iso*thiocyanate was removed by washing several times with benzene. The PTC-derivatives were transferred to the corresponding PTH-amino acids by heating with 1 N HCl at 100 °C for one hour. The PTH-amino acids were extracted and identified by paper chromatography. The solvents A, D, E and F of Edman and Sjöquist [5,6] and a solvent composed of D and E in equal parts [7] were used.

Blombäck and Yamashina [8] have recently reported that there are 2 tyrosyl and 2 glutamyl residues as N-terminal amino acids in the bovine fibrinogen molecule, when the molecular weight was estimated to be 340 000. During fibrinogenolysis the

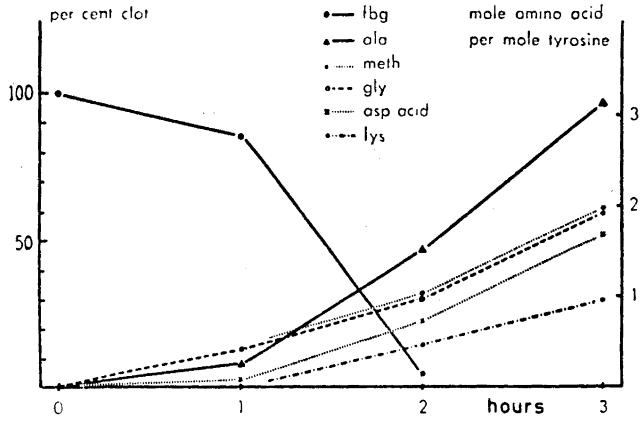

Fig. 1. N-Terminal amino acids released during fibrinogenolysis and correlated to coagulability.

following N-terminal amino acids appeared: aspartic acid, glycine, alanin, lysine and methionine. The glutamyl and tyrosyl residues did not show any significant change during the digestion time used. As the value of tyrosine was practically constant during the fibrinogenolysis, the released amounts of the N-terminal amino acids were expressed in relation to tyrosine (see Fig. 1). When the digestion of fibrinogen had proceeded so far that only traces could be coagulated with trombin, approximately one mole lysyl, two moles each of glycyl, aspartyl and methionyl, and three moles alanyl residues were formed from one mole of fibrinogen.

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An Antibiotic Related to the Streptothricin Group

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From an unidentified actinomycete No. 4279 isolated from a soil sample collected in North Zealand, Denmark, in 1953 we have succeeded in isolating a crude substance with antibiotic activity against a wide range of bacteria and fungi. This substance has properties very similar to those described by Schaffner, Rangaswami and Waksman [1,2] as regards the mycothricin complex though it differs from the latter in certain chemical respects. Optimum yield was obtained after 36 h at 25—28° in a medium containing the extractive material from 40 g of soy-meal per l in an aerated and stirred fermentator.

A crude preparation of the active substance was obtained from the broth by adsorption on activated carbon at pH 7—8 and elution with 80 % ethanol at pH 2.7. Purification was carried out (a) by treating a 20 % aqueous solution of the substance at pH 2.7 with activated carbon and subsequent precipitation by means of 10 volumes of acetone or ethanol and (b) by adsorption on Amberlite IRC-50 at pH 8 and elution with dilute hydrochloric acid; the active fractions were then neutralized with Amberlite IR-4B and evaporated *in vacuo* or, in some cases, lyofilized.

The substance is readily soluble in water and slightly soluble in ethanol. It is insoluble in chloroform, ether, benzene, toluene and *n*-butanol. Paper chromatograms developed with dilute ethanol at pH 3.0 showed the following R_F values: 0.38 (ethanol:water, 80:20), 0.70 (ethanol: water, 70:30), The total activity of the substance can be precipitated by ammonium reineckate, methyl orange, and picric acid, whereas only a fraction of the total activity can be precipitated by flavianic acid (pH between 5.0 and 5.5).

The following tests were negative: Ninhydrin, Pauly, Sakaguchi, Hopkins-Cole, Fehling, Tollens, Molisch, 9-hydrazinoacridin for hydrophilic aldehydes [3]. The biuret reaction was dubious. With sodium periodate the substance gave formaldehyde.

On hydrolysis in 2 N hydrochloric acid an amino acid and a sugar were formed.

Minimum inhibitory concentration

Test organism	μg/ml
Staph. aureus	0.25— 0.31
E. coli	12.5 — 25.0
Strept. hemolyticus	1.0 — 10.0
S. typhi	1.0 — 10.0
Corynebact. sp.	0.1 — 1.0
B. subtilis	0.1 — 1.0
B. dysenteriae Shiga	1.0 — 10.0
Pseud. aeruginosa	10 —100
Alternaria circinans	0.12— 0.25
P. notatum	0.25— 0.50
A. niger	1.0 — 10
P. pullulans	1.0 — 10
Candida albicans	12.5 — 25
Trichoderma viride	0.25— 0.50