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The effect of aliphatic amino acids on the activation of trypsinogen and on the stability of trypsin solutions

ε-Aminocaproic acid is known to inhibit the activation of plasminogen and to a lesser degree the fibrinolytic and esterase activity of plasmin[1–3]. Several other aliphatic amino acids with terminal amino groups also inhibit plasminogen activation, but they are less potent than ε-aminocaproic acid. In descending order of effectiveness they are δ-aminovaleric acid, δ-aminolevulinic acid, ω-aminocaprylic acid and γ-aminobutyric acid[4]. The purpose of this study was to find out whether ε-aminocaproic acid and related compounds would interfere also with the activation of trypsinogen. The amino acids were examined for their effect on the autocatalytic activation of trypsinogen as well as on the activation by enterokinase. Besides ε-aminocaproic acid the series included ω-aminocaprylic acid, δ-aminovaleric acid, γ-aminobutyric acid and β-alanine.

To measure the amount of trypsin formed in the activation mixtures, an assay system had to be chosen which itself would not be affected by the inhibitory substances in the concentrations used. An esterolytic assay with TAME as a substrate was found to fulfil this condition and was employed for all determinations. LME, on the other hand, is less suitable as a substrate because the LME esterase activity of trypsin is inhibited, though only mildly, by ε-aminocaproic acid[2] as well as by δ-aminovaleric acid and ω-aminocaprylic acid (Table I).

The assay procedure was as follows: to 0.4 ml of the solution to be tested (diluted, if necessary, to contain approximately 0.25 mg% trypsin) were added 0.8 ml of

TABLE I

INHIBITION OF LME ESTERASE ACTIVITY OF TRYPSIN BY ALIPHATIC AMINO ACIDS

Incubation mixtures of 1.6 ml contained as final concentrations 0.033 *M* lysine methyl ester, 1.25 mg% trypsin (crystalline, salt-free, Mann Res. Lab.) and 0.1 *M* amino acid in 0.1 *M* Tris buffer (pH 7.6, 37°). Incubation for 45 min at 37°.

Amino acid	*inhibition (%)*
β-Alanine	0
γ-Aminobutyric acid	0
ε-Aminocaproic acid	2.9
δ-Aminovaleric acid	14.3
ω-Aminocaprylic acid	14.9

Abbreviations: TAME, *p*-toluenesulfonyl-L-arginine methyl ester; LME, L-lysine methyl ester.

TABLE II

INHIBITORY EFFECT OF ALIPHATIC AMINO ACIDS ON THE AUTOCATALYTIC ACTIVATION OF TRYPSINOGEN

Incubation mixtures of 2 ml contained as final concentrations 25 mg% trypsinogen (crystallized, Pentex Inc.), 2.5 mg% trypsin, 0.1 *M* $CaCl_2$ (8), and inhibitor, in 0.1 *M* Tris buffer (pH 7.6, 4°) Incubation for 20 h at 4°. At the end of the incubation period, the trypsinogen in the controls was activated 100%.

Amino acid	*Concentration*		
	0.1 M	*0.05 M*	*0.01 M*
	Inhibition (%)		
β-Alanine	59.9	29.4	12.9
γ-Aminobutyric acid	90.5	49.4	29.1
ε-Aminocaproic acid	96.2	87.1	58.0
δ-Aminovaleric acid	100	100	66.2
ω-Aminocaprylic acid	100	100	74.9

0.1 *M* Tris buffer (pH 7.6, 37°), and 0.4 ml 0.065 *M* TAME–HCl in 0.1 *M* Tris buffer. The mixtures were incubated for 30 min at 37° and then the amount of non-hydrolyzed TAME was determined by a modified HESTRIN method[5–7]. Aliquots of 0.4 ml were removed, cooled to room temperature and mixed with 0.4 ml 2 *M* $NH_2OH \cdot HCl$ and 0.4 ml 3.5 *N* NaOH. After 25 min the mixtures were acidified with 0.4 ml HCl (conc. HCl diluted 1:2) and then 5 ml 0.11 *M* $FeCl_3$ in 0.04 *N* HCl (pH 1.2) were added. The absorbancies were read against a blank at 525 mμ. None of the five amino acids interfered with the actual determinations of non-hydrolyzed ester. However, ω-aminocaprylic acid was found to increase the spontaneous hydrolysis of TAME (and LME), and therefore controls containing ω-aminocaprylic acid had to be employed if the concentration of the amino acid in the TAME incubation mixtures exceeded 0.01 *M*. Enterokinase (purified, Pentex Lab.) tested in concentrations up to 75 mg% did not exhibit any TAME esterase (or LME esterase) activity.

The results on the inhibition of trypsinogen activation are summarized in Tables II and III. ω-Aminocaprylic acid and δ-aminovaleric acid were the two most potent inhibitors in the series and compared very closely in their effect on the autocatalytic reaction as well as on the activation by enterokinase. ε-Aminocaproic acid was distinctly less effective than those two amino acids which is in contrast to the findings

TABLE III

INHIBITORY EFFECT OF ALIPHATIC AMINO ACIDS ON THE ACTIVATION OF TRYPSINOGEN BY ENTEROKINASE

Incubation mixtures of 2 ml contained as final concentrations 25 mg% trypsinogen, 25 mg% enterokinase, and inhibitor in 0.1 *M* Tris buffer (pH 7.6, 4°). Incubation for 10 h at 4°. At the end of the incubation period the trypsinogen in the controls was activated 30%.

Amino acid	*Concentration*		
	0.1 M	*0.05 M*	*0.01 M*
	Inhibition (%)		
β-Alanine	2.5	0	0
γ-Aminobutyric acid	26.2	11.7	3
ε-Aminocaproic acid	76.6	63.0	21.0
δ-Aminovaleric acid	100	97.5	69.1
ω-Aminocaprylic acid	100	97.8	68.6

in plasminogen activation[4]. It was also interesting to note that γ-aminobutyric acid and β-alanine showed considerable inhibition of the autocatalytic process but little inhibition of enterokinase. The large amount of enterokinase present in the incubation mixtures probably insured complete or near complete activation, even though the autocatalytic reaction, which will run concurrently once the first trypsin is formed, was suppressed.

The importance of the terminal location of the amino groups for the inhibitory effect[4] was demonstrated by comparing the potency of γ-aminobutyric acid with that of DL-β-aminobutyric acid, DL-α-aminobutyric acid and α-isoaminobutyric acid. At a concentration of 0.1 *M*, γ-aminobutyric acid showed 90% inhibition of the autocatalytic reaction, while the corresponding value for DL-β-aminobutyric acid was 30%, and for both DL-α-aminobutyric acid and α-isoaminobutyric acid only 13%.

TABLE IV

EFFECT OF ALIPHATIC AMINO ACIDS ON THE STABILITY OF TRYPSIN SOLUTIONS

The incubation mixtures of 2 ml contained as final concentrations 25 mg% trypsin and 0.1 *M* amino acid in 0.1 *M* Tris buffer (pH 7.73, 23°). Incubation for 24 h at 23°.

Amino acid added	*Trypsin activity recovered (%)*
None	7
γ-Aminobutyric acid	22.7
β-Alanine	28.0
ε-Aminocaproic acid	47.9
ω-Aminocaprylic acid	53.2
δ-Aminovaleric acid	62.5

The aliphatic amino acids also increase the stability of trypsin solutions as is shown in Table IV, ω-aminocaprylic acid and δ-aminovaleric acid again being most effective. It is suggested that this stabilizing effect may be a simple measure of the inhibitory potency of these compounds on the proteolytic activity of trypsin.

In summary, these experiments have demonstrated that aliphatic amino acids with terminal amino groups inhibit the autocatalytic activation of trypsinogen and the activation by enterokinase. These amino acids also have a stabilizing effect on trypsin solutions.

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