Immunochemical and Kinetic Evidence that Heparin Enhances Aprotinin Activity

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Abstract—Both immunochemical and kinetic evidence suggest that the increase in aprotinin activity in the presence of heparin is not due to an increase in the active form of aprotinin, but rather to a qualitative change in the aprotinin molecule which would lead to an increase in aprotinin activity.

Aprotinin, a basic polyvalent protease inhibitor, is present in mast cells of bovine tissue (1, 2) and is prescribed for patients with acute pancreatitis and shock due to acute peritonitis (3). I reported that preincubation of aprotinin and heparin, the latter a main component of mast cell granules, enhanced the inhibitory effect of aprotinin on the esterolytic activity of trypsin (4). In solution, aprotinin exists in two states, a monomer and a dimer (5–7), and the monomer form is regarded as the active form of aprotinin (8–10). Regarding the enhancing effect of heparin on aprotinin activity, there are at least two possibilities: (a) change in the ratio of the two states of aprotinin and (b) change in the aprotinin molecule which would lead to an increase in aprotinin activity. The present paper reports immunochemical and kinetic evidence for the enhancing effect of heparin on aprotinin activity.

A commercial sample of Trasylol, which corresponds to 1.4 mg of crystalline aprotinin per ml (Bayer, A. G.), served as aprotinin. Heparin, 1,000 IU/ml, was obtained from Novo Industry, trypsin from Miles Laboratories, tosyl-L-arginine methyl ester hydrochloride (TAME) from Peptide Institute, Inc. The inhibitory effect of aprotinin on trypsin activity was measured with TAME as a substrate using the method of Simlot and Feeney (11). Immunochemical titration of aprotinin was as described (12).

The following results were obtained: 1. As shown in Fig. 1, the equivalence points

of both groups at which aprotinin activity is first detected in the supernatant were identical, but the inclination of the curve of the group preincubated with heparin [H(+)] was steeper than that of the group preincubated without heparin [H(–)]. 2. Aprotinin preincubated with or without heparin caused noncompetitive inhibition of trypsin under the present experimental conditions (incubation of enzyme with inhibitor before addition of substrate and short reaction time). In the presence or absence of aprotinin, heparin did not per se have any effect on the K_m value of trypsin for TAME; however, the inhibitor constant (K_i) for the trypsin·aprotinin complex and the trypsin·TAME·aprotinin complex was decreased in the presence of heparin (Table 1).

Gelatin, a hydrophobic protein, also enhanced the inhibitory effect of aprotinin on the esterolytic activity of trypsin (12). The enhancing effect of gelatin was due to an increase in the active form of aprotinin in solution, based on findings such as the following: in the immunochemical titration of aprotinin, gelatin treatment decreased the value of the equivalence point of aprotinin. The present immunochemical result was in marked contrast with that of gelatin. In addition, using the trypsin titration technique, the amount of the active form of aprotinin which reacts with trypsin remained unchanged regardless of the preincubation with heparin (13). Heparin used in this study did not have any effect on trypsin (4) (Table 1,

Fig. 1. Immunochemical titration of aprotinin. Various amounts of aprotinin, with or without heparin in solution, in which the ratio of aprotinin to heparin was 0.14 μg to 8 IU, were prepared using a 0.9% NaCl solution containing 1% benzylalcohol, a solvent used for heparin. The aprotinin solutions with heparin [H(+)] or without heparin [H(-)] were preincubated at 37°C for 48 hr and were then added to equal volumes of antiaprotinin IgG fractions (1 mg/ml) in 0.9% NaCl solution containing 0.01% NaN_3 . The mixture was incubated at 37°C for 4 hr and was allowed to stand at 4°C for overnight. After centrifugation at 30,000 $\times$ g for 20 min, aprotinin activity in the supernatant was measured. The trypsin activity without aprotinin was taken as 100%, and the trypsin inhibition % shown on the ordinate was calculated from the trypsin activity with aprotinin in the supernatant. Each point shows the mean of triplicate assays. The equivalence points of H(+) and H(-) were identical.

Table 1. Kinetic constants of the interaction of trypsin with aprotinin

		K_m (M)	K_{app} (M)	K_i (M)
Heparin	(-)	1.0×10^{-4}	1.0×10^{-4}	6.0×10^{-3}
	(+)	1.0×10^{-4}	1.0×10^{-4}	2.5×10^{-8}

Aprotinin (0.56 $\mu\text{g}/\text{ml}$) was preincubated with or without heparin (32 IU/ml) at 37°C for 48 hr. The preincubation mixture was incubated with trypsin (5 $\mu\text{g}/\text{ml}$) at 37°C for 5 min, and then various amounts of substrate (TAME) were added. The enzymatic activity of trypsin was measured by the method of Simlot and Feeney. The kinetic constants of the interaction of trypsin with aprotinin were obtained from Lineweaver-Burk plots and Hofstee plots of the data. K_m : Michaelis constant of trypsin for TAME in the absence of aprotinin. K_{app} : Apparent Michaelis constant of trypsin for TAME in the presence of aprotinin. K_i : Inhibitor constant for trypsin-aprotinin complex and trypsin-TAME-aprotinin complex obtained in the noncompetitive inhibition.

K_m and K_{app}), while it easily binds to aprotinin in vitro (14). On the other hand, the change in K_i value for the trypsin-aprotinin complex and the trypsin-TAME-aprotinin complex was observed in the presence of heparin. These findings also suggest that the enhancing effect of heparin is not due to an increase in the active form of aprotinin, but rather to some qualitative change in the aprotinin molecule which would lead to an increase in aprotinin activity. Details on the change in the aprotinin molecule induced by heparin remain to be determined.

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