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Activation of Cathepsin D by Glycine Ethyl Ester

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Cathepsin D purified from bovine spleen is activated by glycine ethyl ester. A maximum of 70% activation was observed at a glycine ethyl ester concentration of 0.1 M and at pH 4.5. The activation effect appears to be reversible.

Cathepsin D is an intracellular acid proteinase of wide distribution (Barrett, 1977). Reversible as well as irreversible inhibition of cathepsin D by a variety of compounds has been described (Barrett, 1977; Rakitzis, 1974; Rakitzis & Malliopolou, 1976; Dionyssiou-Asteriou & Rakitzis, 1978). However, no activators of this enzyme are known. In the present communication, the activation of purified bovine spleen cathepsin D by glycine ethyl ester is reported.

Materials and Methods

Cathepsin D (purified from bovine spleen), bovine haemoglobin (type II) and glycine ethyl ester were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Glycine and n-butylamine were obtained from Merck, Darmstadt, W. Germany.

Sodium acetate buffers used were in the pH range 3.0–5.0. The final molarity of the buffer in the incubation medium was 0.1, except for the pH 3.0 samples, where the acetate molarity was 0.4.

Incubation mixtures contained, in a final volume of 5.1 ml: sodium acetate buffer of the required pH, 1% (w/v) haemoglobin, 0.653 μ g of cathepsin D/ml and glycine ethyl ester as required. The final pH of the incubation mixture was determined with a Radiometer 29 pH-meter. The rest of the assay procedure for cathepsin D activity was as described previously (Rakitzis, 1974).

Results and Discussion

The effect of glycine ethyl ester on cathepsin D activity is shown in Fig. 1. Cathepsin D activity increases with increasing glycine ethyl ester concentration, up to 100 mM. At higher glycine ethyl ester concentrations there is a sloping off of cathepsin D activity. The activation effect is most pronounced at pH 4.5. Results from an independent set of experiments, performed with the same glycine ethyl ester concentration in each instance but at different pH values, is shown in Fig. 2. Glycine ethyl ester does not produce a shift in the pH region of the maximum activity of this enzyme.

Fig. 1. Activation of cathepsin D by glycine ethyl ester at pH 3.5 (○), 4.0 (●) and 4.5 (■).

The percentage activation is the activity of cathepsin D in each sample compared with that in the absence of glycine ethyl ester at the pH value used. In the pH 3.5 and 4.5 curves, points are the mean values obtained with three independent experiments. The vertical bars indicate ± 1 S.D.

It was decided to determine whether the activation effect of glycine ethyl ester on cathepsin D is time-dependent. Cathepsin D was incubated at pH 3.85 with 20 mM-glycine ethyl ester and 1% (w/v) haemoglobin. The reaction was stopped after 1 and 2 h of incubation at 37°C. The activation effect, when compared with similarly treated control samples, was the same in each instance (47% activation for the sample incubated for 1 h and 43% activation for the

Fig. 2. Effect of pH on cathepsin D activity in the absence (●) and presence of glycine ethyl ester [10 mM (○) and 100 mM (■)] in the incubation medium

In all cases shown, the incubation time of haemoglobin with cathepsin D was 30 min. For details of the incubation procedure see the text.

sample incubated for 2 h). To determine whether the activation effect is a reversible or an irreversible one, a mixture was prepared containing 0.1 M-sodium acetate buffer, pH 4.5, 0.1 M-glycine ethyl ester and cathepsin D. The mixture was incubated at 37°C for 1 h. At the end of the incubation period, 4 ml of a 1.25% (w/v) haemoglobin solution in 0.1 M-acetate buffer was added, and the incubation was continued for 1 h. Similarly treated samples, without glycine ethyl ester, were used as controls. The activation effect was the same (22%) whether glycine ethyl ester had been added after 1 h of preincubation or with the haemoglobin solution. Had the activation effect been exerted at the preincubation step, i.e. at a

glycine ethyl ester concentration of 0.1 M, the activation would have been 70%. These results indicate that activation of cathepsin D by glycine ethyl ester is neither time-dependent nor irreversible. To determine whether compounds of a structure similar to glycine ethyl ester also have an activating effect, 0.1 M-glycine at pH values of 3.7, 4.0 and 4.5, and 0.1 M-n-butylamine at pH 4.5, were incubated with 1% (w/v) haemoglobin and cathepsin D. No activation effect was observed with either of these compounds.

No activation effect was observed when haemoglobin was incubated with cathepsin D for 1 h, and then glycine ethyl ester was added, immediately before the addition of 0.1 M-trichloroacetic acid. It was established that glycine ethyl ester did not give a blue colour with the Folin-Ciocalteu reagent, neither did it increase the A_{700} because of reaction of haemoglobin-degradation products with the Folin-Ciocalteu reagent. Wojtowicz & Odense (1970) have reported that urea (in the concentration range 1–6 M) increases the solubility of cathepsin D-catalysed haemoglobin-degradation products, and thus produces a spurious activation effect. To test for a similar effect with glycine ethyl ester, this compound was added at 0.1 M to trichloroacetic acid-treated haemoglobin samples that had previously been incubated with cathepsin D. The samples were left at room temperature for 45 min, and then filtered and caused to react with Folin-Ciocalteu reagent in the standard way for cathepsin D determination. No increase in absorbance was obtained as compared with similarly treated controls.

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