DOES ERGOSTEROL INFLUENCE TRYPTIC ACTIVITY?

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1. Introduction

An enzyme–steroid interaction which changes the catalytic activity of an enzyme has been demonstrated in the case of dehydrogenases [1]. In the field of proteolytic enzymes, a recent study [2] has shown that the rate of tryptic hydrolysis of serum albumin can be influenced by the interaction of the substrate with steroids. However, the esterolytic activity of trypsin on synthetic substrates was found not to be influenced by the presence of steroids. Therefore it has been concluded, that the effect of steroids can be attributed to a conformational change in the substrate induced by the ligand [2].

On the other hand, according to earlier work trypsin forms a stable complex with ergosterol and the enzymic activity of this complex against serum albumin and egg albumin as substrates was higher than the activity of trypsin alone [3] indicating that the ligand interacts directly with the enzyme. The results of the above study have been discussed recently by others [4]. Since the observation of a direct influence of a sterol on the catalytic activity of trypsin would be of interest for a better understanding of the mechanism of action both of trypsin and of steroid hormones, we wish to report briefly here on the results of an attempt to reproduce the published study on trypsin activation by ergosterol [3] using a pure enzyme sample (β-trypsin) and more modern methods for following the degree of hydrolysis of substrate.

2. Materials and methods

Crystalline beef trypsin was obtained from Choay (France); β-trypsin was prepared from the crystalline trypsin according to Schroeder adn Shaw [5]. Ergosterol (5.7.22-ergostatrien-3-ol), *N*-α-tosyl-L-arginine-methyl ester and egg albumin were obtained from Fluka. *p*-Nitrophenyl-*p'*-guanidino-benzoate hydrochloride was from Nutritional Biochemicals Co. The Folin–Ciocalteau reagent was from Fisher Scientific Co.

The molarity of active trypsin was determined by active site titration with *p*-nitrophenyl-*p'*-guanidino benzoate at pH 8.3 [6]. The esterase activity of trypsin was assayed spectrophotometrically using *N*-α-tosyl-L-arginine methyl-ester as substrate [7]. Protein concentrations of solutions were determined according to Lowry [8] and spectrophotometrically from their absorbances at 280 nm.

For the formation of a complex between the enzyme and ergosterol the procedure orginally described [3] was adopted. A freshly prepared enzyme solution (3.5×10^{-5} M β-trypsin in 1.10^{-3}N HCl or in 0.06 M phosphate buffer, pH 8.5) was mixed in a mortar with an excess of crystalline ergosterol (10 mg/ml solution) for 10 min at room temperature and the resulting suspension was filtered.

Also the experimental conditions described originally for the digestion of egg albumin by trypsin at different enzyme–substrate ratios [3] were followed.

Egg albumin (a 2% solution in 0.06 M phosphate buffer pH 8.5) was denatured by heating for 10 min on a boiling water bath. Solutions of enzyme and enzyme treated with ergosterol were diluted in such a way as to give successive enzyme–substrate ratios (w/w) from 1:25 up to 1:400. 1 ml of protein solution was mixed with 1 ml of enzyme solution and incubated for 90 min at 37°C. The digestion was terminated by the addition of 3 ml of 5% trichloroacetic acid. The filtrate was kept for 12 hr at 4°C and then its optical density at 280 nm measured.

For the time–dependence study a 1% solution of egg albumin in the same buffer as above pretreated by heating at 100°C for 10 min was digested by β-trypsin in an enzyme–substrate ratio 1:100 at 37°C. At different time intervals 1 ml samples of the digest were mixed with 3 ml of 5% trichloroacetic acid and processed as previously.

3. Results and discussion

When (i) a freshly prepared solution of β-trypsin, (ii) a solution containing the same quantity of enzyme which had been preincubated for 10 min and (iii) a solution resulting from the treatment of the same quantity of β-trypsin with ergosterol (as described in Materials and methods) were assayed for esterase activities and molarities of active site no significant difference was observed between them. This indicates the ergosterol does not activate β-trypsin.

Moreover, the protein contents of the three solutions as determined by the Lowry method were identical indicating that β-trypsin is not absorbed by ergosterol and no difference was found in the absorbances of the three solutions at 280 nm indicating that ergosterol does not form a soluble complex with trypsin.

The degree of proteolysis of egg albumin at different β-trypsin–egg albumin ratios is shown in table 1. The differences between the action of β-trypsin and that of β-trypsin pretreated by ergosterol are within the experimental error.

A study concerning the time-dependence of the comparison of the extent of proteolysis over 4 hr by β-trypsin and by the same quantity enzyme pretreated by ergosterol indicated a slight decrease and not an increase (10%) in the rate of proteolysis by the pretreated enzyme. We have no explanation to offer for this phenomenon.

Table 1
Degree of proteolysis of egg albumin by β-trypsin and β-trypsin pretreated by ergosterol.

Enzyme:substrate ratio (w/w)	Optical density of the filtrate after hydrolysis	
	by β-trypsin	by β-trypsin treated by ergosterol
1:25	1.250	1.200
1:50	0.530	0.540
1:100	0.410	0.400
1:200	0.330	0.340
1:400	0.300	0.270

In conclusion these results indicate that ergosterol does not activate trypsin either for synthetic or protein substrates under conditions, described earlier in the literature [3]. The only explanation we can offer to account for the difference between the present and earlier results is that in the present work β-trypsin has been used, whereas the trypsin used earlier was less well defined. In any case it seems unlikely that the activation of trypsin by ergosterol reported earlier can be ascribed to a trypsin:ergosterol interaction.

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