INTERACTION BETWEEN PEPSIN AND CHYMOTRYPSINOGEN

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Experiments in vitro show that during interaction between pepsin and chymotrypsinogen, the latter is not activated at pH 2.0, 5.0, and 8.0.

Trypsin is the natural activator of chymotrypsinogen. Besides trypsin, many bacterial proteases (from Penicillium, Bacillus subtilis) have an activating action on chymotrypsinogen. Activation of chymotrypsinogen by proteases of Streptomyces griseus has been studied in detail [3]. These proteases have been found to be highly effective activators, their action reaching an optimum at pH 7.5. A study of terminal amino-acid residues has shown that the mechanism of action of the proteases of Streptomyces griseus is identical with that of trypsin. Proteases of Aspergillus oryzae [1] activate chymotrypsinogen just as completely as trypsin. The optimum of action of Aspergillus oryzae is in an acid medium, pH 3.0-5.0. The work of Kaufman and Erlanger [4] has shown that acetylated trypsinogen, under the action of pepsin, is converted at pH 3.2-3.4 into an enzyme with the properties and activity of trypsin.

In the present investigation the action of pepsin on chymotrypsinogen was studied in vitro.

EXPERIMENTAL METHOD

The chymotrypsinogen A used in the experiments was recrystallized 5 times, the trypsin, obtained from fresh bovine pancreas [5], was recrystallized twice, and the hog pepsin was purified on a column with DÉAÉ-cellulose. The solutions were made up in 0.0025 N HCl. The pH was kept constant by means of the Theorell – Stenhagen universal buffer mixture. Activity of chymotrypsin and pepsin was determined by their action on milk-acetate mixture, in samples of 0.5 ml at 35°C [3]. Before determination, the chymotrypsinogen was activated with trypsin at pH 8.0 and 35° for 20 min. The possibility of slow activation of chymotrypsinogen by pepsin at 4° in the proportion of 1000:1 and of its rapid activation at 35° in the proportion of 100:1 at different pH values was studied.

EXPERIMENTAL RESULTS

The results of the action of low concentrations of pepsin on chymotrypsinogen (slow activation) are given in Table 1. The chymase activity, determined in the sample without activation of chymotrypsinogen, never exceeded the activity of pepsin itself. In no experiment was there an increase in activity which could have resulted from activation of chymotrypsinogen. Consequently, only pepsin possessed detectable activity. It follows from these results that pepsin has maximal stability at pH 5.0, and it breaks down rapidly at pH 8.0.

After activation of the samples with trypsin at pH 2.0, during the first minutes of incubation of the mixture the chymotrypsinogen activity fell sharply, and disappeared completely after 1 h. In mixtures with pH 8.0, the decrease in chymotrypsinogen activity was much less marked. At pH 5.0, trypsinogen mixed with pepsin showed maximal stability. Analysis of activity of chymotrypsinogen after activation with trypsin showed that proenzyme was actively destroyed by pepsin at pH 2.0. The decrease in potential activity of the proenzyme in a mixture at pH 8.0 was unconnected with the action of pepsin, because pepsin is inactivated

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TABLE 1. Interaction between Pepsin and Chymotrypsinogen

Incubation time	Activity in activating mixture			Control (chymotrypsinogen without pepsin)	
	pH 2.0	pH 5.0	pH 8.0	pH 2.0	pH 8.0
15 min	0,047 / 0,160	0,047 / 0,330	0 / 0,330	0 / 0,330	0 / 0,330
30 min	0,037 / 0,050	0,050 / 0,030	0 / 0,200	0 / 0,330	0 / 0,200
60 min	0,037 / 0	0,050 / 0,200	0 / 0,083	0 / 0,330	0 / 0,066
24 h	0,010 / 0	0,042 / 0,055	0 / 0	0 / 0,330	0 / 0
48 h	0 / 0	0 / 0,043	0 / 0	0 / 0,200	0 / 0

Note. Numerator gives activity of sample (in units) without activation, denominator gives activity after activation with trypsin. Intrinsic activity of pepsin in sample 0.05 unit. Potential activity of chymotrypsinogen 0.33 unit.

in this medium (figures in numerator), but was due to thermal denaturation. The rate of denaturation corresponded to the rate of denaturation in the control tests. A study of the action of high pepsin concentrations on chymotrypsinogen at pH 2.0, 5.0, and 8.0 showed the same relationship between these enzymes.

LITERATURE CITED

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