Phosphoamidase Activity of some Proteolytic Enzymes and Rennin

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In the course of an investigation of the properties and distribution of phosphoamidase we found that certain crystalline proteolytic enzymes will hydrolyze N(*p*-chlorophenyl)-amidophosphoric acid [1] a substrate of phosphoamidase (Fig. 1.)

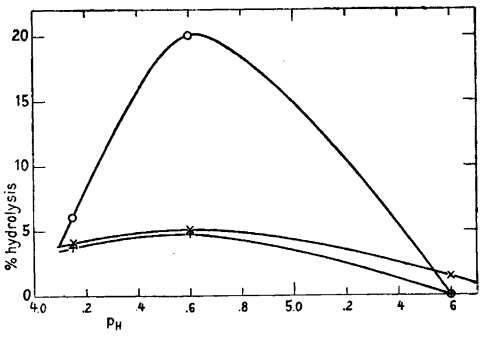

Fig. 1.

The substrate was a 2.52 m*M* solution of N(*p*-chlorophenyl)-amidophosphoric acid in 0.2 *M* acetate buffer. 2 volumes of this solution were incubated with 1 vol. of the enzyme solution for 2 hours at 40°. The enzyme solutions were: 1) 0.1 % (dry weight) Armour crystalline pepsin (0.129 mg N/ml), 2) 0.1 % (wet weight) crystalline trypsin, prepared according to Kunitz (0.0655 mg N/ml); 3) 0.1 % (wet weight) crystalline rennin, prepared by Berridge [2] (0.0535 mg N/ml) * In Fig. 1 the values for rennin are the original ones; the other values have been reduced to the nitrogen value of the rennin solution.

We cannot claim that Fig. 1 represents the correct proportion between the activities of the three enzymes since the samples, although crystalline, were not freshly prepared. However, even if the specific activities shown may be slightly falsified by the presence of inactivated enzyme nitrogen, the graph still indicates that the phosphoamidase activity of rennin far surpasses that of pepsin and trypsin. The optimum pH of 4.6 is the same that we have found for the phosphoamidase activity of various tissue homogenates against the same substrate (unpublished experiments).

To answer the question whether the phosphoamidase activity of the crystalline enzyme preparations might be due to an impurity, we have tried whether the trypsin activity can be inhibited by soybean inhibitor, in analogy to the inhibition of the proteolytic activity of trypsin. This proved to be the case, and therefore it is unlikely, at least in the case of trypsin, that the phosphoamidase activity is an impurity. Schwert, Neurath, Kaufman and Snoke [3, 4] have found that proteolytic enzymes, among them trypsin, exert esterase activity. A similar situation might exist in the present case.

A 2.52 m*M* solution of the phosphoamidase substrate was incubated at pH 4.6 with trypsin in the concentration given above, for 3 hours at 40°. In this time 11.8 % of the substrate was split. The same reagents in the presence of crystalline soybean inhibitor (twice the weight of trypsin) gave a hydrolysis of only 1.3 %.

The fact that phosphoamidase activity is especially high in the case of rennin may help in clarifying the old and unsettled question of the primary action of rennin upon casein. Several investigators [5, 6] have felt that it was by no means certain that the initial chemical change of the casein which induces the process of coagulation is of a proteolytic nature. In 1932 one of us suggested [7] that the initial rennin action might consist in the splitting of P-N bonds in casein. We have tried to study this question by

* The rennin sample was given to us by Dr. H. Nitschmann, Berne, to whom we express our sincere thanks.

comparing the milk-clotting activity of various preparations with their phosphoamidase activity. The result is given in Table 1.

Table 1.

Enzyme prep.	*a* Phosphoamidase activity % hydrol	*b* coagulation R. U.	*b/a*
Rennin	20.7	453	21.9
Pepsin	12.4	262	21.2
Chymotrypsin	8.26	184	22.3
Calf stomach	27.8	189	6.8
Cow stomach	14.7	35.4	2.4

The phosphoamidase activity is given as percentage hydrolysis under the conditions described under Fig. 1. The milk coagulation was measured according to Jacobsen [8] and the activity given in "rennin units" (R. U., according to Berridge [2], only using minutes instead of seconds to designate coagulation time). The rennin and pepsin solutions were the same as employed in the experiment of Fig. 1. Chymotrypsin was a crystalline preparation in 1 % (dry weight) solution *. The calf and cow enzymes were crude homogenates of the mucosa of the 4th stomach, applied in a concentration of approximately 10 % of wet weight.

The remarkable equality of the ratio between coagulating and phosphoamidase activities in the case of the three crystalline enzymes supports the view that there really is a connection between the splitting of P-N bonds and the coagulation of casein. It also supports the view that both activities are inherent properties of the enzyme protein molecule. Regarding the homogenates it must be remembered that in a number of other tissues (spleen, brain, liver etc.) there exists considerable phosphoamidase activity without any milk-clotting power. The same is true of crystalline trypsin. We therefore have to consider the existence of several phosphoamidases of which only one induces milk coagulation. The admixture of non-coagulating enzyme makes itself felt in the mucosa homogenates of calf and cow, in spite of their high content of rennin and pepsin. In trypsin and the tissues mentioned above only non-coagulating phosphoamidases are present and the ratio b/a correspondingly drops to zero. Of course, this phenomenon could also be explained by the assumption that the considerable proteolytic activity of the tissues complicates matters by preventing the milk coagulation which otherwise might be induced.

Finally we should like to mention the possible similarity of the enzyme here investigated and the phosphoprotein phosphatase described by Harris [9] and further investigated by Feinstein and Volk [10]. Their results regarding the distribution of the enzyme in various mammalian tissues correspond roughly to our findings in a similar survey.

This investigation is continued and will be published in greater detail in the *Comptes Rendus du Laboratoire Carlsberg.*

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