THE PHOSPHATE ION AND HYDROLYSIS BY PANCREATIC LIPASE.

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In a recent paper Lyon (1926–27) has used data on the activity of lipase in phosphate buffer solutions of different H^+ and PO_4''' ion concentrations to substantiate a general thesis on the effect of the phosphate ion on certain biological processes. He finds that the results of various workers conform to the equation:

$$(\text{Activity of enzyme})(\text{pPO}_4)^n = K,$$

where pPO_4 is the expression introduced by Holt, La Mer and Chown (1925) to represent the concentration of the PO_4''' ion, and n and K are constants. Certain results obtained by the writers on the effect of phosphates on the hydrolysis of esters by pancreatic lipase conformed to the equation put forward by Lyon. It is found, however, that other data are not amenable to this mathematical treatment. Some explanation of the discrepancies is given in the present paper.

In the experiments to be described, the lipase preparation was obtained from pig's pancreas by the method used by Hewitt (1927) for obtaining undenatured proteins from serum. In view of the explanations advanced of the action of lipase based on the insolubility of this enzyme, it should be noticed that by this means a preparation is obtained which is mainly soluble in water and which can be filtered several times, even through a hardened filter paper, to yield a clear solution without any appreciable loss in activity.

The Effect on the Activity of Lipase of Changing the PO_4''' Concentration by Varying the pH.

According to Lyon the hyperbolic relationship between pPO_4 and the activity of the lipase holds whether the concentration of the PO_4'''

ion be increased by increase of pH or by increase of the total salt concentration. That this is not the case is obvious from a consideration of the results given in Fig. 1 of a previous paper (Platt and Dawson, 1925), where it is shown that the enzyme exhibits optimal activity at about pH 7.0 when 0.05 M phosphate mixtures are used. Thus keeping the total phosphate concentration constant while decreasing the pPO_4 by increasing the pH, the enzyme activity first rises and then

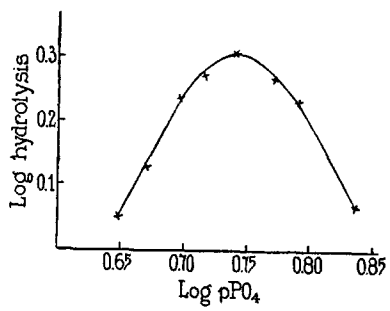

FIG. 1

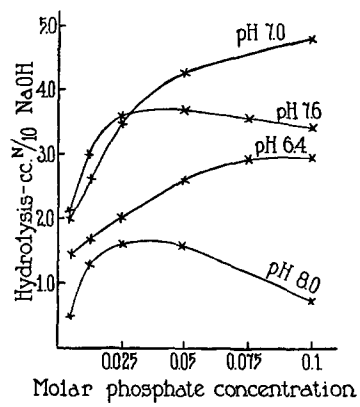

FIG. 2

FIG. 1. Logarithmic plot of the relation between pPO_4 and the amount of hydrolysis of ethyl butyrate. The molar phosphate concentration is the same in all cases, the pPO_4 being altered by adjusting the pH. Experimental details are given in Experiment 2 (Platt and Dawson, 1925).

FIG. 2. The effect of phosphate concentration on hydrolysis of ethyl butyrate by lipase at various hydrogen ion concentrations. The mixtures used contained 10 cc. KH_2PO_4-NaOH buffer mixtures, 2 cc. solution containing 10 mg. of lipase preparation and 0.5 cc. ethyl butyrate. 10 cc. samples were titrated with N/10 NaOH after shaking for 1 hour at 37°C.

falls. These results have been plotted in Fig. 1 below, and show that the activity of the enzyme is optimal at a certain pPO_4.

The Effect on the Activity of Lipase of Varying the PO_4''' Concentration at Constant pH.

If the amount of hydrolysis of ethyl butyrate by pancreatic lipase is determined in solutions of constant H^+ but varying PO_4''' ion con-

centrations, aberrations from the linear relationship occur when the solutions are more alkaline than pH 7.2. This is all the more apparent when the amount of hydrolysis is estimated after a short incubation period, or, in other words, when the values plotted approximate to the initial velocities.

The Effect on Lipase Activity of Treatment with Buffer Solutions of Various PO_4''' and H^+ Ion Concentrations.

The stability of lipase preparations from pig's pancreas varies in buffer solutions of differing H^+ and PO_4''' ion concentration. The

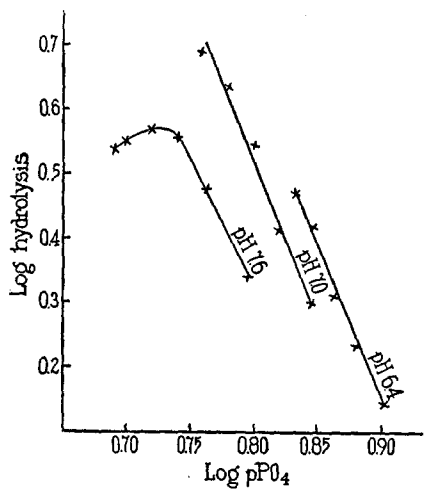

FIG. 3

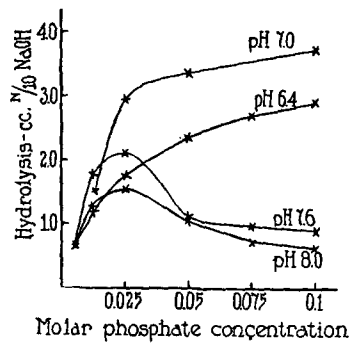

FIG. 4

FIG. 3. The relation between log hydrolysis and log pPO_4, using the values for hydrolysis and PO_4'''ion concentration plotted in Fig. 2.

FIG. 4. The relation between phosphate concentration and amount of hydrolysis of ethyl butyrate in 1 hour at 37°C. by lipase previously treated for 2 hours with phosphate buffer solutions of varying $PO_4'''$and H^+ ion concentrations. The quantities used were the same as those for the experiment reported in Fig. 2 above.

term "stability" is not to be confused with the sensitivity of the enzyme to changes in concentration of PO_4''' ions. The former is determined by the amount of destruction of the enzyme in the presence of PO_4''' or OH' ions, whilst the sensitivity, measured by the constant n of Lyon's equation, represents the degree to which the hydrolytic activity of a particular preparation is promoted by phosphate ions.

An attempt has been made to separate the effect of PO_4''' and OH' ions on the stability from that on the sensitivity of the enzyme. The influence of the ions on the stability can be intensified by a preliminary treatment with the buffer solutions before the addition of the substrate. The activity of the enzyme after treatment can be determined either by immediate addition of equal amounts of substrate or, by first adjusting all the mixtures to the same pH and phosphate concentration. The former method is simple and has usually been followed since essentially similar results are obtained with both methods. Experiments carried out along these lines showed that solutions more alkaline than pH 7.0 have a marked influence on the stability of the enzyme. Still more marked changes are produced by increasing the phosphate concentration in alkaline solutions.

It should be pointed out that for a complete analysis of the reaction, the effect of ions other than those considered above, as well as the influence of the undissociated salt, should be taken into account.

SUMMARY.

The equation, (activity of enzyme) $(pPO_4)^n = K$, has been investigated and has been shown to have only a limited application to the effect of the phosphate ion on the hydrolytic activity of pancreatic lipase. The deviations observed are ascribed to the effect of certain factors on the stability of the enzyme.

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