CHAPTER 11

EFFECT OF pH ON THE ACTIVITY OF LOLIGO PHOSPHORYLASE $\alpha$

The hydrogen ion concentration affects the affinity of an enzyme for its substrate, the maximum rate of reaction and the protein stability. The resultant of these effects is generally a narrow pH range of activity with an optimum pH. The contribution of each of these can be separated and experimentally evaluated relative ly. Construction of Michaelis Menten-Henri profiles and derived plots such as those of Lineweaver and Burk Plot at each pH enables the individual pH - dependencies of $K_m$ and $V_{max}$ to be ascertained. Such plots also allow the analysis of the other phenomena which may arise at certain hydrogen ion concentrations, such as substrate inhibition or activation (157).

Effect of pH on the macromolecular stability of the enzyme can be quantitated by exposing it to different hydrogen ion concentrations for varying times, followed by assay at a fixed intermediate value. For most enzymes, inactivation and denaturation become significant only at pH extremes, where the catalytic activity would be very low. At intermediate values, the environmental hydrogen ion concentration determines the state of ionization of the amino acid side chains in the protein, the enzyme-substrate interaction and the charged state of the substrate.

For the interpretation of the observed pH profile in terms of side chain ionizations at the active site, the bell shaped graph (Fig.11-1) may be treated as the composite of two sigmoidal curves. Consider the left hand 'S' shape ascending from the lower pH values to the optimum pH. This could be taken as corresponding to the ionization of acidic groups in the active site. As the pH was raised this ionised to $R-C=O$. Similarly second sigmoid could be considered to be due to the conjugate acid
(H⁺B) of a basic species (B) at the active site. As the pH increased, concentration of the unprotonated base increased which was inactive, resulted in a progressive decrease in activity. Combination of these two constitutes Fig.11-1.

The rate of inactivation of enzyme, like other proteins is in most cases greatly dependent on the pH of the solution. The effect of pH varies greatly from one enzyme to another. In general there is a zone of maximum stability, not necessarily around the isoelectric point, and the inactivaton increases on the acid and basic sides. Many enzymes are inactivated even at room temperature. Factors other than pH may also have a considerable effect on the inactivation rate, for example ionic strength, protein concentration and the protective action of the substrate, the inhibitors and other substances. The concentration of the water is also important and enzymes are comparatively heat stable. From the previous chapters, it is observed that the Loligo enzyme is not an exception to the above mentioned factors.

Fig.11-1 shows the pH profile of Loligo phosphorylase a. The optimum pH (6.9-7.0) is quite similar to that of rabbit phosphorylase a and b, a little higher than that of liver phosphorylase (pH 6.2-6.4) and a little lower than that of the E. Coli maltodextrin phosphorylase (pH 7.2) (158).

Since the pH profile of Loligo phosphorylase is almost similar to that of Sepia and rabbit phosphorylase (22), the same active groups might be expected to be involved in catalysis. Although the actual nature of the groups in rabbit enzyme has not been unambiguously identified, the 5'-phosphate group of the PLP has been shown to directly participate in catalysis (70-71). The kinetic mechanism of Loligo enzyme is similar to that of rabbit enzyme and the reaction mechanism including effect of pH may be the same.
Fig. 11-1
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*Loligo vulgaris* phosphorylase *a* activity as a function of pH. The enzyme was diluted in 10 mM Sodium-B-glycerophosphate of the required pH and added to the substrate solution containing 16 mM glucose 1-P, 1 mM and 0.5 per cent glycogen adjusted to the required pH. The assay was done at 30°C under standard conditions.