SUMMARY

The thesis deals with the comparative study of glycogen phosphorylase from selected cephalopods, leading from the detailed study of *Lolio vulgaris* Mantle muscle phosphorylase a.

The apparent Km values for glucose-1-P and glycogen of Loligo phosphorylase illustrate the effect of glycogen and glucose-1-P on the affinity. Km values for glycogen remain unaltered at different concentrations of glucose-1-P but Km value for glucose-1-P decreases when glycogen concentration is increased, suggesting that the binding of glycogen enhanced the affinity for glucose. Heterotropic interaction due to the binding of glycogen favoured binding of glucose-1-P but not *vice versa*. The results show inconsistency with the predictions of the model of Monod where allosteric transitions are represented by T and R states and the transitions are affected through changes in the Km values only.

The Kinetic content $K_5$ (i.e., Michael constant of glucose-1-P at saturating glycogen concentration) of Loligo phosphorylase $a$ is higher than *Sepia* and rabbit phosphorylase $a$. The dissociation constant for glucose-1-P ($K_8$) is much less than that in *Sepia pharaonis* and slightly higher than that in *Cibium guttattam*.

The reciprocal plots of AMP are curved downward showing negative cooperativity between AMP binding sites. Similar negative cooperativity has been reported for *Sepia*, *Metapenaeas dobsoni* and Villorita. This negative cooperativity may be an adaptation to the energy need of the animal. Although AMP is the only
natural activator that we have found so far, the physiological significance of the slight shift in Kinetic constant is not clear.

The influence of varying concentrations of Glucose-1-P on Loligo phosphorylase $a$, in the presence and absence of glucose-1-P is not at all comparable with that of rabbit phosphorylase. Apparent Km value of glucose-1-P in the presence of glucose-6-P in Sepia phosphorylase is lower (4) than in the Loligo enzyme (37.5) which implies the low degradative efficiency of Loligo enzyme.

Since the increment in activity due to AMP is more at higher AMP concentration, it is possible that AMP binding brings about a conformational change in the enzyme and that this conformation is less sensitive to glucose. The glucose inhibition in Loligo enzyme is of mixed type which again is different from that in rabbit phosphorylase. It is likely, therefore, that glucose is either competing or interacting with other sites, glycogen binding site is a possible one. However, the effect of glucose, in causing a conformational change, cannot be ruled out.

Paranitrophenol and Paranitrophenyl phosphate increase the activity ratio at all levels of glucose-1-P. This suggests that there is no protection by AMP. So binding of PNP and PNPP in Loligo phosphorylase $a$ is at a site other than the AMP site. Since the binding sites are overlapping, PNP, PNPP, glucose-1-P, AMP and glucose-6-P may bind on the region located near the monomer/monomer interface. This is possible only if the ligands adopt different modes of binding, the modes being determined by their structural features. The inhibition of PNP and PNPP are not time dependent whereas modification by aromatic compounds like FDNB has been shown to be time dependent. These reagents undoubtedly bind on the enzyme prior to reaction.
Studies on temperature effect reveal that charge neutralization and exclusion of water is possible in Loligo phosphorylase. The most likely amino groups in the enzyme sites are lysyl and arginyl residues which are positively charged under assay condition. The activation energy at saturation level of glucose-1-P is 3.69 Kcal/mol which is less when compared to that of saturation levels of AMP (4.35 Kcal/mol). $n$ values of Hill plots in loligo phosphorylase $a$ (saturating levels of glucose-1-P (16 mM) and varying concentration of AMP) are in an increasing order of 0.6, 0.8 and 0.83 at 20, 30 and 35°C respectively. Since the entropy change in loligo phosphorylase is quite small, there may be a loss of water when activated complex is formed.

Cold inactivation of loligo phosphorylase cannot exclusively be attributed to cold temperature because inactivation and hence conformational changes occur under different temperatures and other conditions used for the studies. Based on the observations, different forms of phosphorylase are proposed in a scheme for explaining cold inactivation. Since cold inactivation is high in loligo enzyme, it has a more flexible conformation than other well studied systems. This can be related to the evolutionary status because the cephalopods are far below rabbit and man in evolutionary status, and hence several fine control mechanisms identified as well developed in rabbit and man operate at a much lower level in loligo phosphorylase.

Effect of ionic concentration in the activity of loligo phosphorylase is found to be the same as that in the well-studied rabbit enzyme. 2.5 mM HgCl$_2$ is sufficient to inactivate the enzyme. Activation by Ammonium sulfate and sodium sulfate is predominant in loligo phosphorylase. This is in agreement with X-ray analysis of Barford and Johnson which shows that sulfate ions appear to have worked as an activator in place of phosphate at the active site and at the phosphorylation site at serine 14.
The optimum pH for phosphorylase activity is 6.9-7.0 which 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 *E. coli* maltodextrin phosphorylase (pH 7.2). Since the pH profile of Loligo phosphorylase $a$ is almost similar to that of Sepia and rabbit phosphorylase, the 5'-phosphate group of PLP has been shown to be directly participating in catalysis.

Even though the *Loligo vulgaris* is primitive in evolutionary status, the extremely high energy demand of the mantle muscle for moving with lightning speed is operated by the modified enzyme system of glycolysis. The unique property of cold inactivation, effect of aromatic compounds and other properties suggest the structural difference of Loligo phosphorylase $a$ from other purified phosphorylases studied so far. Considering the functional difference of Loligo enzyme, it is possible to speculate that the specific influence of modified groups in the binding sites promote ATP as an activator at 10 mM level. The metabolic role of ATP seems to be significant as it is found that Loligo muscle is maintaining a very low concentration of AMP. In brief loligo phosphorylase $a$ exhibiting the nature and properties of desensitised rabbit phosphorylase.

A comparative study of glycolytic enzymes from marine sources is given in chapter 13. Glycolysis seems to be the oldest energy yielding process in the biosphere. A fairly narrow spectrum of subunit aggregation is advantageous and thus has been preserved. The phosphorylase $a$ and $b$ interconversion through phosphorylation - dephosphorylation is an excellent example of subunit interaction which is an absolute prerequisite for regulation of enzyme action. A survey of 14 glycolytic enzymes is given in Table 13-5. Of the 14 enzymes surveyed, only phosphoglycerate kinase appears to be a monomer. All the others are dimers or higher multimers, and
moreover with only a few exceptions, a given subunit pattern appears to be quite constant for each enzyme. Most of the glycolytic enzymes have preserved a subunit structure through evolution and the multimeric enzymes seem to be made up of identical or very similar subunits, each containing one active site. Many of the exceptions are found in highly specialised organisms like *Loligo vulgaris*, commonly known as squid.