M.S.22. THOMAS P. THOMAS – Studies on the Enzyme Glucan Phosphorylase – 1982 – Dr. George Philip.

The thesis has two parts. Part I deals with studies on rabbit muscle glycogen phosphorylase and Part II deals with glycogen phosphorylase from the mantle muscle of the marine invertebrate *Sepia pharaonis*.

Part I

Inhibition of the rabbit muscle phosphorylase *a* and *b* was examined using *m* and *p*-nitrophenyl phosphate, p-nitrophenyl ß-glucoside, *p*-nitrophenyl ß-arabinoside, ATP and glucose-6.P. Sigmoidal saturation curves were obtained for phosphorylase *b* in the presence of each of these inhibitors. Inhibition of phosphorylase *a* by the above compounds was of the mixed type. Location of the binding domain in the enzyme was analysed using isobologram. The results indicated that all these ligands bind either mutually exclusively or nearly so on the same location in the enzyme near the monomer/monomer interface where the binding sites of glucose-1-P and AMP are also located. AMP afforded some protection of activity. Nitrophenols were the most effective inhibitors. Based on the degree of inhibition by these inhibitors, a mechanism of different modes of binding by ligands on the same region in the enzyme was presented to explain the special properties of phosphorylase.

The bifunctional reagent 1,5-difluoro-2, 4-dinitro-benzene was used for further studies to locate the aromatic binding site, and to prepare an aromatic derivative of the enzyme. The reaction with phosphorylase *b* was found to be very specific at low concentration of the reagent and resulted in inactivation of the enzyme at a rate much faster than by 1-fluoro-2,4-dinitrobenzene.

Glucose-1-Phosphate, glucose-6-P, AMP and ATP afforded some protection against inactivation by 1,5-difluoro-2,4-dinitrobenzene.

Glucose-1-phosphate, glucose-6-P, AMP and ATP afforded some protection against inactivation by 1,5-difluoro-2,4-dinitrobenzene. The above ligands and aromatic compounds were shown to bind on the enzyme in the same region which is located near the monomer/monomer interface.

An apparently homogeneous dinitrophenylene derivative of phosphorylase *b* with only one group incorporated per dimeric enzyme and having 50% of the catalytic activity was prepared. In the derivative, subunits were not cross-linked. It was devoid of the homotropic cooperativity for the substrate or activator site even in the presence of allosteric inhibitors. Spectroscopic analysis of the derivative showed that one lysyl and one tyrosyl residues were modified, which resulted in the desensitization of the enzyme. The derivative was structually different from the native enzyme as evidenced from its comparatively higher

instability to urea, temperature and the lower reconstitution rate of apodinitrophenylene enzyme. The derivative could be converted to the *a* form like the native enzyme.

Glucose behaved quite differently from other ligands in its effect on inactivation by the reagent and on the kinetics of the modified enzyme derivative. The results showed that glucose, unlike the other ligands did not bind on the aromatic binding site. Also, the binding of glucose was found to cause allosteric transitions.

Rabbit muscle glycogen phosphorylase at higher concentrations was found to effect a slow hydrolysis of *p*-nitrophenyl phosphate and similar nitrophenyl compounds. This effect was established as due to non-specific effect of some amino acid resiudues on the enzyme.

Part II

∝-glucan phosphorylase from specialised tissues of marine and estuarine invertebrates have not been subjected to detailed studies. Glycogen phosphorylase *a* was purified to homogeneity from the mantle muscle of the Cephalopod *Sepia pharaonis*. The mantle tissue is very much similar to the flight muscle of insects. The molecular weight of the phosphorylase purified was about 200,000. Estimation of the pyridoxal-5'-phosphate and the molecular weight suggested that the enzyme was a dimer. The kinetic mechanism was consistent with rapid equilibrium randum *bi bi*, similar to the enzyme from other sources. The kinetic constants in the direction of glycogen synthesis were evaluated.

The *S. pharaonis* phosphorylase was found to be specifically activated by L – cysteine considerably (about 3-fold) unlike phosphorylases from other sources reported so far. Cysteine shifted the 335 nm band of the *Sepia* enzyme to 300 nm. These two effects of cysteine were found to be independent. The effect of cysteine was explained by a specific structural change which conferred an increased hydrophobic character near the PLP site. Such a change should aid in maintaining a fully protonated form of PLP.

The *Sepia* phosphorylase *a* was not inhibited by glucose-6-P, glucose or ATP in the presence of 1 mm AMP, like its rabbit counterpart. However, unlike in rabbit phosphorylase *a*, in the absence of the nucleotide, the *Sepia* enzyme was only slightly inhibited by glucose and not at all by ATP. As with rabbit phosphorylase *a*, aromatic compounds showed mixed inhibition with the *Sepia* enzyme. The enzyme was found to be more sensitive to cold inactivation. Based on the results, a mechanism for 'cold inactivation' was suggested. It may be noted that cold inactivation first observed for the rabbit enzyme has not yet been satisfactorily explained.

The control mechanism of the glycogen phosphorylase from *Sepia* was found to be different from other animal muscle phosphorylases. The *Sepia* enzyme exhibited negative homotroic cooperativity between AMP site. Fresh *Sepia* muscle contained active phosphorylase phosphatase and kinase which became inactivated on freezing of the muscle. Rabbit muscle phosphorylase phosphatase and kinase did not interconvert the *Sepia* phosphorylase suggesting structural differences. The animal was found to maintain a very high concentration of AMP (about 3.5 mM) in its mantle tissue. The high energy demand of the mantle was met by this high concentration of AMP such that enzyme would be fully active whether it was present in the *a* or *b* form. Therefore interconversion between AMP-dependent and AMP-independent forms of phosphorylase did not appear to be a s major controlling factor of glycogen degradation, unlike in other cases studied so far.