M.S.53. SOSAMMA CHERIYAN–Glycogen Degrading Enzymes and their regulation in Benthic Animals and Estuarine Fish–1985–Dr. George Philip

The major control of glycogen degradation at the enzyme level is by glocogen phosphorylase in animals. To study the regulation of glycogen degradation in different aquatic animals, form different species were selected. They are *Etroplus suratensis*, a brackish water fish, *Metapenaeus dobsoni* an estuarine cum marine prawn and two bivalves *Sunetta scripta* (marine) and *Villorita cyprenoides* (estuarine). Among them *M. dobsoni* occupies the lowest evolutionary status and the bivalves are in between the fish and *M. dobsoni.*

Phosphorylase activity per gram tissue were found specific activity in the original extract were found to vary in the different animal tissue extracts in a definite manner consistent with the energy requirement of the tissues. The stability of the enzymes (measured by activity loss) in the extracts and at different stages of purification were found to correlate well with the evolutionary status of the animal. ie, higher the animal's status, the more stable the enzyme.

Phosphorylase was purified either completely or partially from the muscle of each of these species and the kinetic properties were studied. The presence of phosphorylase phosphatase and phosphorylase kinase were also tested in all these animals and also their action on crystalline rabbit muscle phosphorylase. From these it was found that both phosphorylase kinase and phosphatase were present in these animals like the other animals and hence the interconversion reaction plays an important role in the regulation of glycogen degradation in these animals also. Only the *M. dobsoni* muscle was found to have a latent form of phosphorylase phosphatase.

The specific activity and activity per gram of phosphorylase in the muscles of the different animals were different, the lowest being found for the bivalves. Activity ratios (activity in the presence of 1 mM AMP to that in its absence) were 0.23 and 0.3 for *Sunetta* and *Villorita* respectively.

Ammonium sulphate and sodium sulphate activated the *Sunetta, Villorita* and *Entroplus* phosphorylases. Heavy metals inhibit all the enzymes. Sodium chloride and potassium chloride had no activating or inhibiting effect. It was also found that except for *Etroplus* phosphorylase the other three phosphorylases were highly unstable, *N. dobsoni* being the least stable.

The kinetic analyses gave data consistent with rapid equillibrium mechanism for all phosphorylases studied. In the case of glycogen/glucose-1-P kinetics, except *Etroplus* phosphorylase the other three phosphorylases showed positive heterotropic cooperativity between the two substrate sites whereas *Etroplus* phosphorylase showed negative heterotropic cooperativity. Quantitatively the three phosphorylases were again different. Kinetics with the activator AMP was different for different species. *Villorita* and *M. dobsoni* phosphorylases showed negative

homotropic cooperativity between AMP sites, *Etroplus* phosphorylase showed negative heterotropic cooperativity between glucose-1-P and AMP sites and *Sunetta* phosphorlase showed positive heterotropic cooperativity between them.

The metabolites ATP, glucose-6-P and glucose inhibited all the four enzymes though the pattern of inhibition was different. For *Sunetta, Villorita* and *Etroplus* these metabolites *a*, in the absence of AMP, mixed typeof inhibition was observed. In the presence of AMP, for *M. dobsoni* phosphorylase *a* glucose-6-P induced negative homotropic cooperativity between glucose-1-P sites. Another difference observed was that while glucose was the most potent inhibitor (among the three metabolites mentioned) for the fish phosphorylase, glucose-6-P was found to be the most potent inhibitor for the invertebrate phosphorylases.

The properties of the four animal phosphorylases were compared with respect to each other and with other well-studied animal muscle phosphorylases. Thus the interconversion between the *a* and *b* forms is a general mechanism of regulation of glycogen degradation in these animals also. Moreover the allosteric regulation by the metabolites glucose, glucose-6-P and ATP and activator AMP is also involved in the control of degradation of glycogen. Comparison of the properties of the phosphorylases reveals that the properties of the phosphorylases reveals that the properties of the enzyme and its control can be explained by two factors *viz.* the evolutionary status of the animals and the energy requirement of the muscle.