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Introductory remarks to the second session

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The four presentations in this session cover studies on different aspects of enzyme structure and function. They effectively illustrate how one has to combine different approaches to arrive at an understanding of enzymatic catalysis and control. Nowadays, the molecular description of an enzyme is hardly credible without detailed crystallographic information. Thus, Dr Blake's paper on the structure of phosphoglycerate kinase is particularly relevant to our understanding of phosphate-transfer mechanisms. The question of the relation between structure in the solid and solution is still with us and n.m.r. has proved to be the best way to study the differences and similarities. Many enzyme substrate complexes have been 'mapped' in solution, by using the perturbation of the n.m.r. spectra by paramagnetic centres as a measure of interatomic distances. How such results can lead to both structural and mechanistic information will be discussed by Dr Mildvan.

To understand mechanisms we must also get some information about the nature of transition states. Here, stereochemical observations play an important role and Dr Lowe will describe some elegant work on the use of chiral phosphates in approaching this problem. Finally, it is important to describe structures, intermediates and transition states in terms of the kinetic behaviour of the enzyme and Dr Dalziel will give us an example of both steady-state and pre-steady-state rate studies. Measurements of reaction rates ultimately link studies on the isolated enzyme to their behaviour *in vivo*. As this last step in the sequence is not covered by the formal presentations at this meeting I should like to show briefly how n.m.r. can now be used to obtain fluxes of enzyme catalysed reactions *in vivo* both in the steady state and at equilibrium.

In collaboration with Professor Wilkie, Dr Dawson, Dr Chance, Dr Gadian and Dr Brown (Gadian *et al.* 1981), we have studied the creatine kinase reaction in isolated frog muscle both at rest and during contraction. Several of the phosphate-containing metabolites can be quantitatively measured by ^{31}P n.m.r., and the activity of creatine kinase in an intact frog muscle can be studied by saturation transfer n.m.r.

In an anaerobic muscle at 4 °C the relevant reactions are

$$\text{phosphocreatine} + \text{ADP} \underset{f_2}{\overset{f_1}{\rightleftharpoons}} \text{ATP} + \text{creatine} \xrightarrow{f_c} \text{ADP} + \text{P}_\text{i}$$

(the charges and the involvement of Mg^{2+} and H^+ are omitted for simplicity of presentation).

At rest, the contractile flux (f_c) is zero, while f_1 and f_2 are approximately equal at 1.6 mM s^{-1}. Creatine kinase is in equilibrium, as it must be, under these conditions. During contraction the creatine kinase reaction is no longer at equilibrium and there is a net breakdown of phosphocreatine, which can be estimated directly from the P_i and phosphocreatine resonances.

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The observed fluxes are as follows: $f_1 = 1.6, f_2 = 0.85$ and $f_c = 0.75$ mM s^{-1}. The important observation is that *in vivo* the creatine kinase reaction is not a great deal faster than the flux at which ATP is used up by the contractile energy demand, yet the ATP concentration remains constant during contraction. The simple trick by which this is achieved is to retain the same forward (f_1) flux from phosphocreatine as at rest, but to reduce the back flux (f_2) to about half of its resting value. How is this possible? The answer lies in the relatively large change in ADP concentration during contraction and the formation of the creatine kinase–ADP–creatine abortive ternary complex. We have been able to show that because of the formation of this complex during contraction, the activity of creatine kinase is inhibited, but nevertheless the forward flux is maintained since the inhibition is compensated for by the increased availability of ADP as a substrate. Thus the detailed interplay between the different enzyme–substrate complexes is the key regulator in this instance.

The experiments described here demonstrate how we can study the properties of enzymes in intact tissues and relate them directly to their known solution behaviour. With this background we can proceed with the next session.

Reference (Radda)

Gadian, D. G., Radda, G. K., Brown, T. R., Chance, E. M., Dawson, M. J. & Wilkie, D. R. 1981 The activity of creatine kinase in frog skeletal muscle studied by saturation transfer nuclear magnetic resonance. *Biochem. J.* **194**, 215–228.

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