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General Discussion

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General discussion

R. J. P. Williams, F.R.S. (*Inorganic Chemistry Laboratory, Oxford, U.K.*). In the light of the frequent references at this meeting to the involvement of phosphorylation in modulating calcium triggering it is of interest to ask how this can come about when the sites of calcium binding and those of phosphorylation are far apart and often on different proteins. I shall attempt to answer this question on the basis of n.m.r. work by Dr B. A. Levine and myself.

We know that calcium binding is to proteins of the EF-hand type, i.e. troponin-C and calmodulin. Calcium, as a positively charged ion, binds negatively charged loops of these helical proteins and subsequently the helical structures of the proteins are adjusted. We have shown that all the proteins behave in a very similar manner. The helix adjustments act as the transmission device for information (energy) from one end of a helix to another. These devices are well described by very detailed n.m.r. studies that we have summarized and will report in detail elsewhere. (Dalgarno, D., Klevit, R., Levine, B., Scott, M. & Williams, R. J. P. 1983 In *Ciba Foundation Symposium* no. 93 (*Mobility and function in proteins and nucleic acids*. (In the press.)) Professor A. Lesk (M.R.C. Molecular Biology Unit, Cambridge) and ourselves have developed general ideas as to how information is transmitted in other helical proteins.

I turn now to phosphorylation. Where the site of phosphorylation is known it is usually on a serine or threonine in a somewhat mobile segment of the protein, e.g. near a terminus, and the residue is often close to positive charges of lysine or arginine. A particular example is found in phosphorylase as mentioned by Professor E. G. Krebs when referring to the work of Dr L. Johnson (Oxford). Perhaps in a very general sense the phosphate group is a negative 'calcium' ion with two major differences: (*a*) the binding is covalent and needs catalysed binding and removal; (*b*) the rate constants for phosphate reaction are about one hundredth of those for calcium. It is presumably to allow enzymic attachment that the phosphate acceptor is often a loose protein segment near a terminus. We can now ask does this binding act on a helical secondary structure as in the calcium proteins and then connect to the calcium trigger? There is the distinct possibility that this is true for phosphorylase. If so, and where it is connected to calcium binding, not only does calcium binding change with phosphorylation but phosphorylation may be affected by calcium binding.

The time constant differences are interesting in that they allow a memory of events of increasing length from electrical (Na^+/K^+) to electromechanical (Ca^{2+}) to chemical-bond mechanical (P) to chemical compound syntheses (C-bond) to develop. Each step can have feedback loops to sustain or shorten (modulate) both earlier or later events. As well as acting back on calcium triggers, the phosphorylation acts forward to other mechanical devices and to metabolism and synthesis. There is a hint of how this might operate in the allosteric effect of phosphate compounds on haemoglobin. Once again the action is through the adjustment of helical segments of the protein.

The adjustment of helical stretches of proteins after binding to exposed loops could be a very general transmission device useful in gating, of calcium channels for example, as well as in control of ATP-utilizing reactions and pumps. The structures of kinases have helical segments next to the hinge regions. Control of a hinge can then be related to helix motion and it is relatively easy to see how phosphorylation could gate the channels of calcium pumps.