Amino-acid Sequence of Tropomyosin

from a Correspondent

FAST progress is now being made in determining the amino-acid sequence of the fibrous proteins. The whole of the sequence of the α_1 chain of collagen is available, though dispersed throughout the literature; now the sequence of about half of the tropomyosin molecule has been reported (Sodek *et al.*, *Proc. US Nat. Acad. Sci.*, **69**, 3800 ; 1972).

There are only four principal groups of fibrous proteins—the α-proteins, the silks or β-proteins, collagen and the rubber-like proteins. The α-proteins make up the keratin-epidermin-myosin-fibrinogen group discovered by Astbury to share a common molecular conformation. The X-ray diffraction patterns obtained from these proteins were interpreted by Crick as indicating that the conformation consists of two α-helical strands wound about each other to form a coiled-coil. Crick noted that the amino-acid composition of tropomyosin shows that two-sevenths of the amino-acids are apolar and he suggested that the α-helices might wind round each other in order to bring regularly occurring apolar groups into contact. Two-sevenths is the correct fraction to permit this.

Tropomyosin is the shortest of the α-proteins and it is thought to be all in the α-helical conformation. The molecules are about 400 Å long and they occur in the thin filaments of muscle where they lie in end-to-end contact in the grooves of the two strand helix of actin. Tropomyosin, in conjunction with troponin, participates in the regulation of muscle contraction.

Sodek *et al.* used the technique of cleaving the tropomyosin molecule into fragments by cyanogen bromide which selectively hydrolyses the peptide bond adjacent to methionine. The sequence of the fragments was determined by further proteolytic cleavage and gel filtration and ion-exchange chromatography of the products; a sequence of about 140 amino-acids in the —COOH terminal half of the chain was obtained. When this sequence was inspected for features of possible structural significance a most pleasing fact emerged. Apolar amino-acid side-chains occur regularly separated alternately by two and three other types of residue. The authors alternatively describe the regularity as two series of apolar residues, one displaced by three residues with respect to the other. In each series an apolar group occurs every seventh residue. An α-helix has 3.6 residues per turn and if the apolar groups are identified they can be seen to lie on the locus of a helix of long pitch round the surface of the α-helix. If two α-helices are tilted with respect to each other so that the long helices are in contact and the projections of one fit into the holes of the other, they may then coil around each other so that the long helices unwind and lie in parallel contact as the central core of a two strand rope.

Some consideration was given by Sodek *et al.* to the efficiency of packing of the two strands in the rope. They report that if the strands are laid in register they pack satisfactorily but not optimally because the large residues come opposite the large and the small opposite the small. Best packing and a more uniform rope radius are predicted if one strand is staggered with respect to the other by fourteen residues. This would imply tail sections with one strand jutting out at each end of the molecule.

BLOOD PLATELETS

Release Reaction

IN a new model the release of calcium ions from blood platelets induced by thrombin is shown to involve at least four steps. The first of these is a rapid binding of thrombin to platelets; this is then followed by a first-order reaction transformation of this complex, a thrombin-independent, slower first-order release of Ca^{2+}, and, finally, a slow turnover of thrombin leading to the release of additional Ca^{2+}. This model is suggested by Detwiler and Feinman (*Biochemistry*, **12**, 282 ; 1973), who have studied the kinetics of this reaction in an attempt to clarify the reaction of thrombin with platelets and the role of Ca^{2+} in platelet function.

Platelets play an important part in haemostasis, which involves the release of constituents such as adenine nucleotides, 5-hydroxytryptamine and Ca^{2+}. It has been suggested that Ca^{2+} may be the agent that mediates other changes. The most potent physiological stimulator of platelets is the enzyme thrombin. Detwiler and Feinman suggest that there is a rapid association between enzyme and platelets and that a critical number of thrombins per platelet is required for the release of Ca^{2+}. A model in which one thrombin reacts with one site with the release of a certain number of moles of Ca^{2+} can be rejected on the basis of reaction kinetics. Thus there can be small amounts of thrombin in circulation without any reaction with platelets.

A second important feature of the studies of Detwiler and Feinman is the observation that there is very little turnover of thrombin; the enzyme is tightly bound throughout the reaction. Binding on the platelet surface seems to be the most likely form, but binding within the membrane or of thrombin transport cannot be excluded. It does seem, however, that binding does not involve the active site of the enzyme, but rather its site of fibrinogen binding. Detwiler and Feinman estimate that a platelet binds up to 2×10^4 thrombins.

CHLOROPLASTS

Phosphorylation

from our Photosynthesis Correspondent

ELECTRICAL gradients can help to drive phosphorylation in isolated chloroplasts, according to Schuldiner, Rottenberg and Avron (*FEBS Lett.*, **28**, 173 ; 1972). This observation has important implications in understanding primary energy conversion in photosynthesis.

Peter Mitchell has presented (*Biol. Rev.*, **42**, 445 ; 1966) an attractive but controversial hypothesis suggesting that a hydrogen ion gradient created across the thylakoid membranes of chloroplasts by light-induced electron flow acts as the precursor to ATP synthesis. This idea was supported by the finding of Uribe and Jagendorf (*Proc. US Nat. Acad. Sci.*, **55**, 170 ; 1966) that when isolated chloroplasts are subjected to a *p*H shift from about 4.5 to 8.5 in dark conditions they synthesize ATP. It was found that gradients of at least 4 *p*H units are necessary to induce significant phosphorylation.

Because, however, hydrogen ions are charged, then the energy available in the *p*H gradient may be governed not only by the concentration difference across the membrane but also by any electrical gradient that may exist. It is indeed still not clear whether both electrical and concentration gradients contribute to the high energy state in illuminated chloroplasts. Although Schuldiner *et al.* cannot answer this question they indicate that electrical and *p*H gradients combined can drive phosphorylation.

Schuldiner *et al.* carried out experiments which were essentially similar to those of Uribe and Jagendorf. They induced changes in *p*H either artificially in the dark or by using low light intensities, but unlike the earlier experiments from Jagendorf's laboratory, they developed hydrogen ion gradients too small to induce high yields of ATP (2.5 to 2.8 *p*H units). In these conditions of limiting proton gradients they were able to show a significant increase of ATP yield when high concentrations of KCl, together with valinomycin, were added to the suspensions.

Schuldiner *et al.* argue that the establishment of KCl gradients across thylakoid membranes treated with valinomycin generates a diffusion potential of a positive polarity inside and that this potential, together with the *p*H gradients, drives ATP synthesis.