Structural resemblance between Schramm's repolymerised A-protein and tobacco mosaic virus

SCHRAMM has shown that rod-shaped particles of the protein of tobacco mosaic virus (TMV) freed from ribonucleic acid (RNA) may be prepared by breaking down the virus in weak alkali, separating the protein by electrophoresis, and subsequently repolymerising the protein by lowering the pH[1]. In this way, a protein of molecular weight about 100,000, which SCHRAMM has called A-protein, can be built up into particles of form similar to that of the original virus.

Good X-ray fibre-diagrams of repolymerised A-protein have now been obtained, and show unequivocally that the reconstituted rods have a structure closely similar to that of the protein part of TMV. The greater part of the diagram of orientated gel of repolymerised A-protein is indistinguishable from that of the complete virus. The two diagrams are compared in Fig. 1.

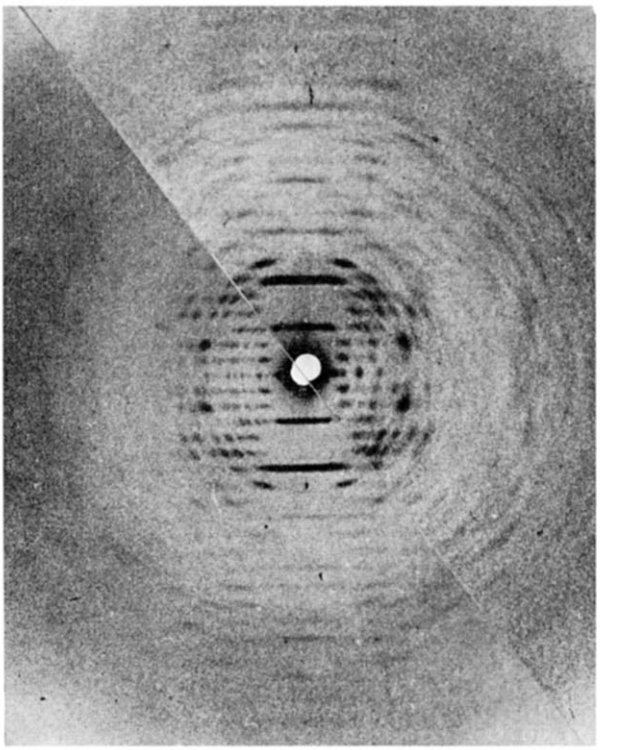

Fig. 1. X-ray fibre diagram of orientated gel of repolymerised A-protein (bottom left) compared with similar diagram given by the TMV from which the A-protein was prepared (top right).

The distribution of intensity on the *equator* of the diagrams is, however, very different in the two cases. This means that the axial projection of the electron density of the particles is different. This is to be expected since the RNA forms a central core in TMV[2,3], and the repolymerised A-protein therefore presumably has a hollow, or water-filled core. Since the structure of the protein is clearly closely similar in the two substances, the difference in equatorial intensities must be directly related to the structural arrangement of the RNA in the virus. Quantitative measurements of equatorial intensities for the two substances are therefore being made with a view to obtaining information about the structure of the RNA.

Two other differences between the X-ray diagrams of repolymerised A-protein and of TMV are observed. In the former the intensity maxima fade out more rapidly at large angles (this is *not* due to less good orientation), and the central regions of the 3rd and 6th layer-lines show stronger streaking along the layer-lines and less pronounced sharp maxima. Both these effects may be attributed to a small degree of disorder in the structure of the polymerised A-protein.

In spite of the streaking mentioned above, the maxima close to but not on the meridian, which are characteristic of the helical structure of TMV protein[4,5,6], are clearly visible in the repolymerised A-protein. Thus it appears that a helical arrangement of the protein sub-units about the particle axis persists even in the absence of the RNA core.

The repolymerised A-protein shows a much more highly ordered structure and a much closer resemblance to TMV than does the B8 protein examined by FRANKLIN AND COMMONER[7]. The latter is a repolymerised protein prepared from an abnormal protein of low molecular weight (B_3) found in the sap of plants infected with TMV[8]. The repolymerised A-protein resembles B8, however, in certain important respects. The gel has a lower positive birefringence than TMV gel of the same concentration, while the birefringence of dry orientated polymerised A-protein is weakly *negative*. This shows once again (*cf.* ref. 7) that the RNA makes a *positive* contribution to the birefringence of TMV, and therefore has a structure very unlike that of DNA, which under most conditions[9], is strongly negative.

Repolymerised A-protein also resembles B8 in that it shrinks longitudinally on drying. It differs from B8, however, in *not* expanding to a length greater than that of TMV when in the gel state. In repolymerised A-protein gel the axial repeat period is 69 Å, as in TMV. In dry orientated A-protein the axial repeat is about 62 Å, and the structure shows a lower degree of order. It seems, therefore, that when the nucleic acid core is replaced by water the structural arrangement of the protein in the virus particle remains stable, but when this water is removed by drying the particle shrinks and becomes partially disordered.

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D(−)β-Hydroxybutyryl CoA dehydrogenase*

The second oxidative step in the sequence of reaction during fatty acid oxidation has been shown to be catalyzed by a DPN-linked dehydrogenase (I) specific for the L(+)β-hydroxyacyl CoA derivatives[1,2].

Recently STERN *et al.*[3] have reported on an enzyme that racemizes D(−) to L(+)β-hydroxybutyryl CoA. They found no effect of added DPN on this racemization. However we were able to obtain a fraction (II) from beef liver mitochondria that catalyzes a DPN specific oxidation of the D(−)β-hydroxybutyryl CoA. The reaction was measured spectrophotometrically by the increase at 340 mμ or 303 mμ due to the formation of DPNH or acetoacetyl CoA[4] respectively. Therefore the name L(+)β-hydroxybutyryl CoA dehydrogenase is proposed. The product of such dehydrogenation has been identified as acetoacetyl CoA by its absorption at 303 mμ in presence of Mg[4,5,6] and by the formation of equivalent amount of citrate in the presence of CoASH, β-keto cleavage enzyme[4,5,7] oxalacetate and condensing enzyme[8]. When D(−)β-hydroxybutyryl CoA was incubated with this fraction in the absence of added DPN^+, there was very little conversion to L(+)β-hydroxybutyryl CoA (as shown by assay with L(+)β-hydroxyacyl CoA dehydrogenase). On addition of catalytic amounts of DPN ($1 \cdot 10^{-5}$ *M*) (but not TPN), conversion could be obtained. In the experiments shown in Fig. 1 D(−)β-hydroxybutyryl CoA was incubated with 100 μg of enzyme and $1 \cdot 10^{-5}$ *M* DPN in sample A and without DPN in sample B. After 30 minutes at 38°, the reaction was stopped by heating, and the mixture assayed for L and D isomers by DPN^+ reduction in the presence of I and II. First I was added at zero time and an equilibrium was obtained which represents the amount of L formed from the D isomer. With sample A which contained DPN^+ during the preincubation, the amount of L formed is approximately 50% of the original D isomer used. On addition of II, the remaining 50% could be accounted for. With sample B the L-isomer produced is considerably less. The effect of catalytic amounts of DPN on the racemization can further be shown (*cf.* Fig. 2) by the formation of the L from the D isomer, with and without added DPN^+ at varying amounts of enzyme. Since the amount of the L isomer formed exceeds htat of the DPN added, this apparent racemization is catalyzed by DPN. L(+)β-hydroxyacyl CoA dehydrogenase is still a contaminant of preparations of II. Thus the apparent racemization can be explained in terms of the following reactions:

$$\text{D(−)β-hydroxybutyryl CoA} + DPN^+ \overset{\text{II}}{\rightleftharpoons} \text{Acetoacetyl CoA} + DPNH + H^+ \overset{\text{I}}{\rightleftharpoons} DPN^+ + \text{L(+)β-hydroxybutyryl CoA}$$

II has an optimum pH at 9.0 and is 85% inhibited by *p*-chloromercuribenzoate at concentrations of $2.5 \cdot 10^{-4}$ *M* while L(+)β-hydroxyacyl CoA dehydrogenase is not inhibited at this concentration. Full activity can be restored by addition of glutathione. When the enzyme was preincubated for 15 minutes at 0° with Co^{++} at concentration of $2 \cdot 10^{-4}$ it would give 100% acti-