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-

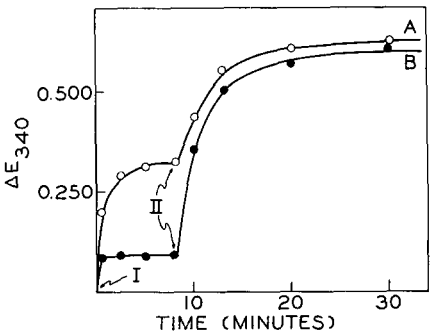

Fig. 1. Samples A and B each contain 20 μmoles of Tris pH 8.5, 100 μg of enzyme, 0.04 μmole of D(—)β-hydroxybutyryl CoA with 0.0015 μmole of DPN in sample A and without DPN in B, and H_2O to 0.1 ml. At the end of the incubation the reaction was stopped by heating, then 0.5 μmole of DPN, 1 μmole of Mg^{++} was added and H_2O to 0.45 ml. The mixture was adjusted to pH 9.0 with alkali, was centrifuged and 0.3 ml was pipetted into a cuvette. The L(+)β-hydroxybutyryl CoA was assayed by addition of 10 μg of L(+)β-hydroxyacyl CoA dehydrogenase at I. After equilibrium was reached 50 μg of D(—)β-hydroxybutyryl CoA dehydrogenase was added at II. Values corrected for two blanks without substrate and without enzyme.

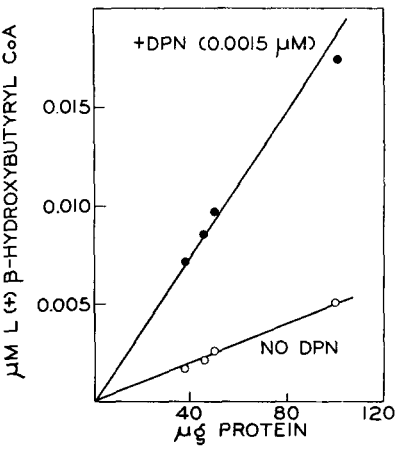

Fig. 2. The apparent racemization of D(—)β-hydroxybutyryl CoA at varying amounts of enzyme with and without added DPN. Conditions were the same as in Fig. 1 except for the amounts of enzyme.

vation of II as measured by the rate of DPNH formation. Other metal ions such as Mg^{++}, Zn^{++}, Cu^{++}, Fe^{++}, Fe^{+++}, Mn^{++}, Ca^{++}, and Cd^{++} do not give such activation. The K_m for the reaction D(—)β-hydroxybutyryl CoA with II is $3.3 \cdot 10^{-5}$ M.

Some preparations of the enzyme obtained by different methods do not require added DPN for the racemization and do not show an increase in the rate of racemization on the addition of DPN even after treatment with norite and Dowex 1-Cl. This observation is similar to that reported by STERN and his associates.

With the aid of this enzyme, it was possible to resolve the problem of the specificity of the unsaturated acyl CoA hydrase with regard to crotonyl CoA (*trans* form) and isocrotonyl CoA (*cis* form). The results show that the product of hydration of the crotonyl CoA is oxidized by DPN in the presence of L(+)β-hydroxyacyl CoA dehydrogenase, while the product of *iso*crotonyl CoA is not acted upon by this enzyme. On the other hand in the presence of D(—)β-hydroxybutyryl CoA dehydrogenase the product of hydration of *iso*crotonyl CoA is oxidized by DPN. This was further supported by the isolation of the free acids after hydrolysis of the CoA derivatives. Only the product of hydration of *iso*crotonyl CoA reacts in the β-hydroxybutyric dehydrogenase system described by GREEN *et al.*[9]. Full details of the specificity of hydrase will be published elsewhere.

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