INTERMEDIATE- AND LONG-CHAIN β-HYDROXYACYL-ACP DEHYDRASES FROM E. COLI FATTY ACID SYNTHETASE[1]

Michinao Mizugaki, A. C. Swindell,[2] and Salih J. Wakil
Department of Biochemistry
Duke University Medical Center
Durham, North Carolina 27706

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Available evidence indicates that β-hydroxydecanoyl-ACP[3] is a common intermediate in the synthesis of both saturated and unsaturated fatty acids in certain bacteria. This intermediate can be dehydrated to α,β-decenoyl-ACP which is then reduced to decanoyl-ACP and elongated to palmityl-ACP; or it can be dehydrated to the β,γ-decenoyl-ACP which is elongated to palmitoleyl-ACP or cis-vaccenyl-ACP. In support of this hypothesis, Bloch and his coworkers (Brock et al., 1967 and Kass et al., 1967) have isolated an enzyme which dehydrates the β-hydroxydecanoyl thioester derivatives of ACP, CoA and NAC[3] to the corresponding β,γ-decenoyl derivatives. Moreover, Pugh et al., (1966) have obtained from the fatty acid synthetase of E. coli, a subfraction (E_{II}) which catalyzed the formation of β-hydroxydecanoyl-ACP. The latter derivative could then be converted to either palmitate or cis-vaccenate upon the addition of two other fractions. As a result of these studies, Pugh et al., (1966) postulated the existence of several β-hydroxyacyl-ACP

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[2]Postdoctoral Fellow of the National Institutes of Health.

[3]Abbreviations: Acyl carrier protein, ACP; N-acetyl cysteamine, NAC; and p-chloromercuribenzoate, pCMB.

dehydrases having different chain length specificities. Recently, Mizugaki et al., (1968) have isolated an enzyme (β-hydroxybutyryl-ACP dehydrase) from extracts of E. coli and found it to be specific for the short chain length β-hydroxyacyl-ACP derivatives (C_4 to C_8); it was inactive on β-hydroxydecanoyl-ACP. Birge et al., (1967) have described, in an unsaturated fatty acid auxotroph of E. coli, an enzyme which catalyzed the dehydration of β-hydroxydecanoyl-ACP to the α,β-decenoyl-ACP; however, no further information was available regarding its chain length specificity.

In this communication we wish to report the presence of two new dehydrases in extracts of E. coli. β-Hydroxyoctanoyl-ACP dehydrase was specific for the ACP derivatives of β-hydroxy fatty acids of chain length C_6, C_8, C_{10} and C_{12}, and had highest activity on the C_8 derivative. β-Hydroxypalmityl-ACP dehydrase was active with the ACP derivatives of β-hydroxy fatty acids of chain length C_{12}, C_{14} and C_{16}, and had highest activity on the C_{16} derivative.

Materials and Methods: The assay method of Mizugaki et al., (1968) was employed for both dehydrases in the presence of ACP substrates. In this procedure, trans-2-enoyl-ACP's were incubated with dehydrase, $MgCl_2$ (.02 M), Tris-HCl (0.1 M, pH 8.5), TPN^+ (0.7 μmole), and excess β-ketoacyl-ACP reductase, in a volume of 0.50 ml. The formation of the Mg complex of β-ketoacyl-ACP was measured by the increase in absorption at 303 mμ.

The β-ketoacyl-ACP reductase was purified according to Toomey and Wakil (1966), and was free of dehydrase or enoyl-ACP reductase activities.

Trans-2-enoyl-ACP substrates were prepared by the procedure

of Weeks and Wakil (1968), from previously acetylated ACP (Birge et al., 1967). Each preparation was assayed by the enoyl reductase method of Weeks and Wakil (1968).

The NAC substrates were prepared by the mixed anhydride method from pure DL-β-hydroxy acids. The enzyme-catalyzed formation of 2-enoyl-NAC was measured by the increase in absorption at 263 mμ, as described by Kass et al., (1967).

β-Hydroxyoctanoyl-ACP dehydrase was purified from the protein fraction E_{IV}, which was obtained by fractionating crude extracts of E. coli on a DEAE-cellulose column according to the procedure of Pugh et al., (1966). The E_{IV} protein (440 mg) was adsorbed on a second DEAE-cellulose column (2.5 x 40 cm). The column was washed with .005 M potassium phosphate, and 10-ml fractions were collected and assayed with 2-octenoyl-ACP. Active fractions (tubes 25-30) were pooled. The protein was precipitated with ammonium sulfate (90% saturation), collected by centrifugation, and redissolved in a minimum volume of phosphate buffer. A sample of the enzyme (in 0.5 ml) was filtered through a column of Sephadex G-100 (1.2 x 20 cm), with .01 M potassium phosphate, pH 7.4; peak enzyme activity emerged at 1.3 x V_o.

The β-hydroxypalmityl dehydrase was purified from the DEAE-cellulose fraction designated E_{III} by Pugh et al. (1966). The E_{III} protein (22 mg in 3.0 ml) was chromatographed on a Sephadex G-100 column (2.5 x 80 cm) and eluted with .01 M potassium phosphate, pH 7.4, containing 5 mM 2-mercaptoethanol. Dehydrase activity was monitored with 2-hexadecenoyl-ACP; peak activity was eluted at a volume of 3.0 x V_o. Active protein was precipitated with ammonium sulfate, collected by centrifugation, and redissolved in 0.01 M potassium phosphate, pH 7.4, containing 5 mM of 2-mercaptoethanol. The preparation was then heated at 70° for

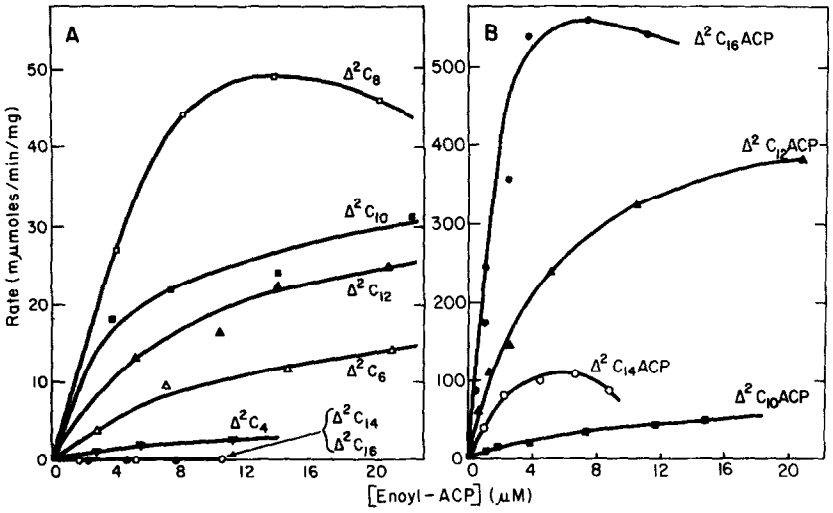

Figure 1. Initial Rates of Enzymatic Hydration of 2-Enoyl-ACP Substrates. The enzymes were: A, β-hydroxyoctanoyl-ACP dehydrase; and B, β-hydroxypalmityl-ACP dehydrase.

15 minutes, and the denatured protein was removed by centrifugation.

Results and Discussion

The β-Hydroxyoctanoyl-ACP Dehydrase: Final preparations of the β-hydroxyoctanoyl-ACP dehydrase were 60-fold purified over crude extracts and had a specific activity of 89 mμmoles/min/mg of protein with 2-octenoyl-ACP as substrate. Under the assay conditions, the formation of β-ketooctanoyl-ACP from 2-octenoyl-ACP was dependent upon the dehydrase, the β-ketoreductase, and TPN^+. As shown in Fig. 1A, the enzyme preparation catalyzed the hydration of Δ^2-ACP derivatives of C_4, C_6, C_8, C_{10} and C_{12} with estimated V_{max} values of 4, 19, 89, 31 and 41, respectively. The enzyme had no activity on the Δ^2C_{14} or Δ^2C_{16} ACP derivatives. Thus the enzyme was specific for intermediate chain 2-enoyl ACP

derivatives and is different from the β-hydroxydecanoyl-ACP dehydrase described by Bloch and coworkers (Brock et al., 1967, and Kass et al., 1967). The latter enzyme was specific for the C_{10} derivative, and its relative activities on the β-hydroxyacyl-NAC thioesters of C_8, C_{10} and C_{12} were 1.5:21:1, respectively.

The β-hydroxyoctanoyl-ACP dehydrase preparation, had relatively low activity with NAC thioester substrates. At 100 μM, the β-hydroxyoctanoyl- and β-hydroxydecanoyl-NAC were dehydrated at rates of 0.8 and 13.8 mμmoles/min/mg protein, respectively; β-hydroxydodecanoyl-NAC, at 30 μM, was dehydrated at a rate less than 0.05 mμmole/min/mg. The ratio of the rates was essentially the same as those reported for β-hydroxydecanoyl-thioester dehydrase, suggesting that the latter enzyme may be a contaminant of the

TABLE I.

Inhibition of β-Hydroxyoctanoyl-ACP Dehydrase by p-Chloromercuribenzoate

The dehydrase (3.8 μg for ACP substrates and 9.5 μg for the NAC substrate) was preincubated for 20 min with pCMB plus the complete assay mixture except substrate. Substrates were preincubated separately with the same pCMB concentration. The two solutions were mixed to initiate reactions. Final substrate concentrations (μM) were: 4.2 (Δ^2C_8-ACP); 3.2 (Δ^2C_{10}-ACP); 7.8 (Δ^2C_{12}-ACP); and 100 (β-hydroxy-C_{10}-NAC). Values in parentheses represent the initial rates of (mμmoles/min/mg protein) reaction in the absence of pCMB.

pCMB conc.	Percent inhibition with substrates			
	Δ^2C_8-ACP	Δ^2C_{10}-ACP	Δ^2C_{12}-ACP	β-Hydroxy-C_{10}-NAC
(M) none	0(23.9)	0(11.8)	0(16.8)	0(13.8)
10^{-5}	24	17	9	9
10^{-4}	91	85	75	11

β-hydroxyoctanoyl-ACP dehydrase preparation. The contribution by this contaminant to the hydration of 2-decenoyl-ACP was estimated to be about 25% of the total observed activity.

The β-hydroxyoctanoyl-ACP dehydrase has an active thiol group as evidenced by its inhibition by 10^{-4} M pCMB[3] as shown in Table I. With the β-hydroxydecanoyl-NAC thioester derivative, however, the inhibition was only 10%, indicating that the residual activity of the enzyme on the NAC substrates was apparently due to the presence of the β-hydroxydecanoyl-thioester dehydrase, which is a pCMB-insensitive enzyme (Kass et al., 1967).

The β-hydroxyoctanoyl-ACP dehydrase was relatively heat-stable; 20 to 30 percent of its activity was lost after heating for 5 minutes at 60 to 65°. However, heating at 70° for 5 minutes resulted in a loss of about 90 percent of its activity. In this regard the β-hydroxyoctanoyl-ACP dehydrase was similar to the β-hydroxydecanoyl thioester dehydrase but different from the dehydrase activity reported by Birge et al., (1967). Whether this difference is due to differences in the E. coli strains used or to the presence of a different dehydrase, remains to be determined.

The β-Hydroxypalmityl-ACP Dehydrase: The enzyme was purified about 520-fold over crude extracts of E. coli, employing 2-hexadecenoyl-ACP as substrate. The assay of such preparations was absolutely dependent upon the presence of enzyme, substrates, β-ketoacyl-ACP reductase and TPN^+. The enzyme preparation was specific for ACP thioester derivatives and was active on 2-dodecenoyl-ACP, 2-tetradecenoyl-ACP and 2-hexadecenoyl-ACP as shown in Fig. 1B. The maximal activities (V_{max}) of the enzyme on the ACP derivatives of Δ^2C_{12}, Δ^2C_{14} and Δ^2C_{16} were 440, 270 and 1330 mμmoles/min/mg of protein, respectively. Relatively lower

activity with the 2-tetradecenoyl-ACP was consistently observed with several enzyme and substrate preparations. No satisfactory explanation for this observation is apparent at this time. The possible presence of more than one dehydrase in this fraction is under investigation.

Preparations of the β-hydroxypalmityl-ACP dehydrase were sensitive to sulfhydryl reagents when activity was measured with each of the three substrates. Fifty percent inhibition of enzyme activities was achieved with 1.2×10^{-6} M pCMB or with 1.2×10^{-5} M N-ethylmaleimide.

The substrate specificities of the three dehydrases for β-hydroxybutyryl-ACP, β-hydroxyoctanoyl-ACP and β-hydroxypalmityl-ACP, encompass the entire chain length spectrum of the intermediates in the synthesis of long-chain fatty acids. β-Hydroxydecanoyl-ACP was recently shown to accumulate in incubations containing β-hydroxybutyryl-ACP dehydrase and other enzymes of a reconstituted fatty acid synthetase system (Mizugaki et al., 1968). The accumulation of β-hydroxylaurate, β-hydroxymyristate and palmitate, observed in subfractions of fatty acid synthetase by Pugh et al., (1966), may be explained by the presence or absence of the two dehydrases described in this report. The restricted specificities of these enzymes may also be important in the synthesis of β-hydroxymyristic acid, a component of cell wall lipids, or in the control of fatty acid synthesis in E. coli.

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