Acyl Carrier Protein

XVII. PURIFICATION AND PROPERTIES OF $\beta$-HYDROXYACYL ACYL CARRIER PROTEIN DEHYDRASE*

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SUMMARY

$\beta$-Hydroxyacyl acyl carrier protein (ACP) dehydrase has been purified 2900-fold from extracts of Escherichia coli. The enzyme catalyzes the reversible dehydration of $\beta$-hydroxyacyl-ACP thioesters to yield specifically trans-2-enoyl-ACP products. It is active with trans-2-enoyl-ACP thioesters of chain lengths from 4 through 16 carbon atoms. The enzyme catalyzes the hydration of cis-5-trans-2-dodecadienoyl-ACP, an intermediate in unsaturated fatty acid synthesis, as well as the trans-2-enoyl-ACP substrates that are intermediates in saturated fatty acid synthesis. Thus this enzyme can function in the synthesis of both saturated and unsaturated fatty acids. The lowest enzyme activity was noted with trans-2-decenoyl-ACP. The relevance of this finding with respect to the activity of $\beta$-hydroxydecanoyl thiester dehydrase, which specifically catalyzes the conversion of $\beta$-hydroxydecanoyl-ACP to cis-3-decenoyl-ACP is discussed as a possible factor in control of saturated versus unsaturated fatty acid synthesis. Attempts to separate or to show distinguishing characteristics of $\beta$-hydroxyacyl-ACP dehydrase activities with short, medium, or long chain substrates were unsuccessful, and these activities are therefore attributed to a single enzyme.

The dehydrase (or dehydrases) responsible for the production of trans-2-enoyl-ACP intermediates of fatty acid synthesis in Escherichia coli are not yet entirely understood. Several enzymes have been reported, but none has been obtained pure. Therefore clear delineation of substrate specificity has not been achieved (1-4). The preceding paper (5) and Reference 6 contain the results we have obtained in examining $\beta$-ketoacyl-ACP synthetase and $\beta$-ketoacyl-ACP reductase for substrate specificity, particularly with regard to the ability to utilize precursors of both saturated and unsaturated long chain fatty acids. In the cases tested, neither of these enzymes discriminated between substrates on the basis of the presence or absence of a cis double bond. We have continued these studies, undertaking the purification of a dehydrase active in the hydration of cis-5-trans-2-dodecadienoyl-ACP, an intermediate in the synthesis of unsaturated fatty acids. Studies of the dehydrase were expected to show that this enzyme was specific for either precursors of long chain unsaturated fatty acids or that its specificity included medium chain length precursors of both unsaturated and saturated fatty acids. In the first case, a unique type of substrate specificity would have been shown. However, the second result was obtained since the purified $\beta$-hydroxyacyl-ACP dehydrase has a broad substrate specificity, including intermediates of both the saturated and unsaturated fatty acid biosynthetic pathways. The properties of the enzyme were studied so that the relationship between this enzyme and the $\beta$-hydroxydecanoyl thiester dehydrase (7-11) in the synthesis of saturated versus unsaturated fatty acids might be understood.

The work of Bloch et al. (7-11) established that both saturated and unsaturated fatty acids are synthesized from acetyl-CoA and malonyl-CoA via a common intermediate, $\beta$-hydroxydecanoyl-ACP. The dehydration of this intermediate, catalyzed by the $\beta$-hydroxydecanoyl thiester dehydrase, leads to the formation of cis-3-decenoyl-ACP which is a specific precursor in unsaturated fatty acid biosynthesis. Alternatively, the dehydration of $\beta$-hydroxydecanoyl-ACP to form trans-2-decenoyl-ACP leads to the synthesis of saturated fatty acids. Thus $\beta$-hydroxydecanoyl-ACP is the intermediate at the branch point between the pathways to saturated and unsaturated fatty acids. Although $\beta$-hydroxydecanoyl thiester dehydrase cannot catalyze the conversion of $\beta$-hydroxydecanoyl-ACP to form trans-2-decenoyl-ACP in vitro (7,8), it apparently does not produce this intermediate in vivo. This was shown by the isolation of an unsaturated fatty acid auxotroph, L010, which lacked the $\beta$-hydroxydecanoyl thiester dehydrase (12). Although this mutant was unable to synthesize unsaturated fatty acids, it produced saturated fatty acids normally. Thus it was apparent that E. coli must contain another $\beta$-hydroxyacyl-ACP dehydrase which catalyzes the $\alpha,\beta$-dehydration of $\beta$-hydroxydecanoyl-ACP to form trans-2-decenoyl-ACP, the intermediate of the saturated fatty acid biosynthetic pathway. Preliminary experiments were...
reported from this laboratory which showed the presence in the
mutant extract of an enzyme which catalyzed the \( \alpha, \beta \)-dehy-
dration, but not the \( \beta, \gamma \)-dehydration, of \( \beta \)-hydroxydecanoyl-
ACP (2). Since in wild type \( E. \text{coli} \) this dehydrase and the \( \beta \)-hy-
droxydecanoyl thioester dehydrase both catalyze reactions
involving \( \beta \)-hydroxydecanoyl-ACP, it appeared likely that control
of the relative amounts of saturated and unsaturated fatty acids
synthesized by \( E. \text{coli} \) could be achieved through regulation of one
or both of these dehydrases. However, although \( \beta \)-hydroxy-
decanoyl thioester dehydrase is stimulated about 2-fold by 0.3
m inorganic phosphate, extensive experimentation has failed to
show regulation of this enzyme by other small molecules, by
changes in temperature at which the assay was performed, or
by changes in temperature at which the cells were grown (7).
Thus it was hoped that availability of the pure \( \beta \)-hydroxyacyl-
ACP dehydrase, which catalyzes \( \alpha, \beta \)-dehydration of saturated
and unsaturated long chain fatty acid precursors including \( \beta \)-hy-
droxydecanoyl-ACP, would allow an evaluation of the role
of this enzyme in the regulation of saturated and unsaturated fatty
acid synthesis.

EXPERIMENTAL PROCEDURE

**Bacterial Strains and Growth Conditions**—The unsaturated
fatty acid auxotroph L010 is a derivative of \( E. \text{coli} \) K-12 and
has been previously described (12, 13). Cultures of 200 liters
were grown in a Fermenta model F-250 fermentor (New Bruns-
wick Scientific Co.). The minimal medium used was described
in the preceding paper (5). Glycerol, 2 mg per ml, was supplied
as growth supplement. Cells were harvested in late logarithmic
growth phase at an optical density of 0.7 to 0.8 (650 nm). Cul-
tures were checked for revertants; none was found to contain
more than 5 in 10^10 cells. The cell paste was stored at \(-20^\circ\).

\( E. \text{coli} \) B, late log phase, was purchased from Grain Processing
Corp., Muscatine, la.

**Enzyme Assays**—\( \beta \)-Hydroxyacyl-ACP dehydrase, which catal-
yzes Reaction 1, was assayed by measuring the rate of this
reaction in the forward or reverse directions.

\[
\begin{align*}
\text{R-CH_2-C} & \quad \text{S-ACP} \\
\text{OH} & \quad \text{S-ACP}
\end{align*}
\]

The dehydration of \( \beta \)-hydroxyacyl-ACP substrates was followed
by measuring the increase in absorbance at 263 nm due to the
formation of trans-2-enoyl-ACP. The molar extinction co-
efficient of trans-2 unsaturated thioesters is 6.7 \times 10^5 (14). The
assay solution, 0.1 ml, contained 10 \( \mu \)moles of Tris-HCl, pH 9.0, 2 \( \mu \)moles of MgCl_2, 0.1 \( \mu \)mole of TPN, 30
units (nanomoles of acetoacetyl-ACP reduced per min) of \( \beta \) ke-
toacyl-ACP reductase, and 5 to 10 \( \mu \)moles of trans-2-enoyl-ACP.
The assay was started by the addition of enough enzyme to give
rates of 0.005 to 0.05 OD unit per min. This assay could not be
used with enzyme preparations which contained high concen-
trations of phosphate or sulfate since these form insoluble mag-
nesium salts which interfere with the optical assay. The assay
was particularly suited for measuring the enzyme activity in
crude dehydrase preparations in which background absorbance
at 263 nm was high. The coupled assay and the simple hydra-
lation assay were compared periodically during purification, and
comparable specific activities were noted with both assays.
Units of \( \beta \)-hydroxyacyl-ACP dehydrase activity are expressed as
nanomoles per min.

Two marker enzymes, catalase, assayed by the method of
Beers and Sizer (16), and hexokinase, measured according to
the method in “Biochemica Catalogue” of Boehringer-Mann-
heim, were used to calibrate gel filtration chromatography col-
umns. Both enzymes were obtained from Sigma Chemical Co.,
Saint Louis, Mo.

All optical density measurements for enzyme assays were
made on a Gilford Recording Spectrophotometer, model 240.
Protein determinations were carried out by the method of Lowry
et al. (17), or by a modification of the biuret reaction (18).

**Preparation of Substrates**—The synthesis of cis-5-\( \beta \)-hydroxy-
dodecanoyl-ACP and of other acyl-ACP substrates was pre-
sented in detail in the preceding paper (5) as were the sources
of all fatty acids.

**Polycrylamide Gel Electrophoresis**—The standard analytical
method of polycrylamide gel electrophoresis according to the
method of Davis (19) was used. Gels of 10% were prepared and
run at pH 9.5. When 8 m urea was included in the system,
solid urea was added to the gel solution. Samples were diluted
into and dialyzed against the standard Tris-glycine buffer con-
taining 8 m urea and 0.01 m dithiothreitol. The electrode buffers
did not contain urea (20).

When material was to be eluted from gels, 2-mm slices were
made and put into small dialysis bags to which was then added
0.2 ml of the usual enzyme buffer. The slices were crushed
within the bags and dialyzed against the same buffer to equi-
librium. The liquid was then removed from the bags with a Hamilton syringe, the volume noted, and assays performed.

Analysis of Fatty Acids—Methylation and gas-liquid-radiochromatography of methyl esters was described under "Experimental Procedure" of the preceding paper (5).

RESULTS

Purification from K-12, L010

The availability of cis-5-trans-2-dodecadienoic acid, generously provided by Doctors L. Heslinga, H. J. J. Pabon, and D. A. van Dorp (21) made possible the routine use of cis 5 trans 2 dodecadienoyl-ACP in either the coupled assay or the hydration assay throughout the purification. The assays were described under "Experimental Procedure." The fab A mutant, L010, was chosen as the source of the enzyme since this organism lacks the β-hydroxydecanoyl thioester dehydrase which might interfere with measurements of the β-hydroxyacyl-ACP dehydrase in crude extracts.

All of the purification steps were carried out at 4°. Before breaking, the cells were washed with 0.1 M Tris-HCl, pH 7.4. The cells were resuspended in this buffer plus 5 mM dithiothreitol and 1 mM EDTA, broken in a French pressure cell at 25,000 p.s.i., and the resulting suspension was centrifuged at 48,000 × g for 1 hour. The supernatant solution, "Crude Extract," was fractionated by ammonium sulfate precipitation. The solid salt was added to 45% saturation while stirring the extract, and the stirring was continued for 30 min. The precipitated material was removed by centrifugation and discarded. The supernatant was brought to 70% saturation with ammonium sulfate precipitation. The solid precipitate was collected by centrifugation and sequentially back extracted with solutions of the buffer which were 60, 55, 50, 45, and 40% saturated with respect to ammonium sulfate. The fractions were dialyzed against buffer. The highest enzymatic specific activities were obtained in either the 55 or the 50% fraction.

It was noted early in the purification process that exposure of the enzyme preparation to ionic strengths lower than that of 0.1 M potassium phosphate buffer caused aggregation and sedimentation of large amounts of activity. The process was reversible in that dialysis of the precipitated enzyme against 0.1 M phosphate buffer resolubilized the protein and full activity was recovered.

During the purification the preparations were stored at 4°, but the purified enzyme was kept in liquid nitrogen where it was stable for several months. A summary of the purification procedure is given in Table I. The scheme was followed through three preparations, starting with 100 to 165 g of frozen cells, with virtually identical results in specific activity and recoveries.

It should be mentioned that the specific activity given for the final preparation, about 12,000 moles per min per mg, represents a conservative figure. Immediately after the final back extraction with ammonium sulfate and several hours of dialysis against buffer, all of the active fractions were found to be about twice as active as they were after 24 to 48 hours at 4°. Thus a rapid loss of activity occurred during this time. The preparations maintained the lower activity, falling off only gradually with storage, and thus the lower figure is reported. Since this phenomenon occurred each time the preparation was carried out, attempts were made to find conditions in which the purified enzyme would not lose activity. Conditions that were tested included storing the enzyme in the usual buffer plus either 10% ammonium sulfate or 20% glycerol or in 1 M potassium phosphate buffer, but none of these stabilized the enzyme.

In addition, the specific activity of a given enzyme preparation was dependent on the substrate preparation used. Although the same batch of ACP and fatty acid were used as starting

![Table I](http://www.jbc.org/)
materials for the synthesis of substrates throughout these studies, differences in specific activity were observed when new preparations of substrate were compared with older ones, presumably due to deterioration of the substrate.

Product of Dehydration Reaction

cis-5,β-Hydroxy[2-14C]dodecenoyl-ACP, synthesized enzymatically as described in the preceding paper (5), was used as substrate to show the product of the β-hydroxyacyl-ACP dehydration reaction. Seventy nanomoles of the thioester, 2.5 × 10^5 dpm, were incubated with 2 μg of enzyme, specific activity of 10,000 counts per min per μg, in 1 ml of 0.1 M phosphate buffer, pH 7.3. The solution was incubated at room temperature until no further increase in optical density at 263 nm was observed; then it was acidified and extracted with ether to remove any free fatty acids produced by hydrolysis of the thioester during incubation. Only 3% of the 14C was extracted under these conditions. KOH was added to the solution to a final concentration of 0.5 M, and hydrolysis was allowed to proceed at 34°C for 1 hour. The pH was then lowered to about 2 with HCl, and the solution was extracted four times with equal volumes of ether. Seventy-five per cent of the 14C was extracted into the ether. The ether extract was washed with water and allowed to evaporate overnight at 35°C. After being methylated, the material was dissolved in CS2 and analyzed by gas-liquid-radiochromatography. The tracing is shown in Fig. 1. The substrate, which slightly preceded β-hydroxydodecanoate used as a reference compound, was converted to a new substance which cochromatographed with authentic cis-5-trans-2-dodecadienoate. The extent of the conversion, by quantitation of the 14C in the peaks, was 40%. A similar analysis of the substrate, similarly hydrolyzed but without exposure to the dehydrase, can be seen in the preceding paper (5), Fig. 5A.

Substrate Specificity

Of major importance was the question of whether the dehydrase would prove to be specific for precursors of the unsaturated fatty acids found in E. coli. Thus, at several stages during the purification of the dehydrase the activity was tested with trans-2-dodecenoyl-ACP, an intermediate in the synthesis of saturated fatty acids. It became apparent that the activity of the enzyme for this substrate was comparable to the activity with the unsaturated precursor and that the activities for these two substrates were overlapping.

A second important question concerned the chain length specificity of the dehydrase. It became clear in the early stages of purification that the hydration of trans-2-decenoyl-ACP was very slow relative to the hydration of either of the substrates mentioned above. It was possible, then, that the dehydrase was active only with substrates longer than 10 carbon atoms.

Once the protein was highly purified, a series of substrates was prepared in order to examine the specificity of the enzyme. A large single batch of previously acetylated ACP was used for all of the acylation reactions, much attention being paid to maintaining identical conditions in the preparation of each thioester. This was felt to be important since considerable variation in enzyme activities has been observed in this laboratory and in others when different preparations of a given acyl-ACP substrate have been used. This variability is considered to be attributable to the variation in preparative procedures, including the possible acylation of nonthiol groups of ACP (22), the extent of removal of excess reactants and solvents, the purity of all components of the reactions, and the ages of the acyl-ACP preparations. Details of the synthesis and the sources of the starting materials are given under “Experimental Procedure” of the preceding paper (5). The substrates prepared and tested were the trans-2-unsaturated acyl-ACP derivatives of 4, 6, 8, 10, 12, and 16 carbon atoms, cis-3-decenoyl-ACP, and cis-5-trans-2-dodecadienoyl-ACP. cis-5-trans-2-Dodecadienoyl-CoA was also synthesized. Data were collected on the initial velocities versus substrate concentrations for each thioester, and these were analyzed in the form of Lineweaver-Burk plots. The data are summarized in Fig. 2, in which the Vmax and Km of each substrate are plotted according to chain length. The enzyme preparation used was 3,000-fold purified and had a specific activity of 12,000 nanomoles per min per mg or better in the standard assay. It was quite surprising to find that the activity, although quite low with the 10-carbon substrate, increased progressively with the 9-, 8-, and 4-carbon thioesters. Lower, but significant activity was seen with the 16-carbon substrate. Although there is reason to have confidence in the relative maximal velocities of these substrates with respect to each other, it should again be emphasized that, because of the variability observed from one preparation of a given substrate to another, the absolute numbers may be only approximate. Three different preparations of trans-2-decenoyl-ACP were tested with the enzyme, however, and none of these gave a higher Vmax value than is reported here. All of the ACP thioesters tested were found to have an apparent Ks within the range of 4 to 8 × 10^−3 M, as indicated in the top half of the figure.

The dehydrase was tested with cis-3-decenoyl-ACP to deter-
Fig. 2. Summary of kinetic data of β-hydroxyacyl-ACP dehydrase. Extrapolation of Lineweaver-Burk plots was used to determine maximum velocities and apparent \( K_m \) values for each substrate.

Properties of β-Hydroxyacyl-ACP Dehydrase

In light of previous reports showing separable short and medium chain β-hydroxyacyl-ACP dehydrase activities (3, 4), it became important to establish whether the broad substrate activity profile illustrated in Fig. 2 was the result of a single or more than one dehydrase in the preparation. Several studies of the physical and chemical properties of the enzyme were conducted with two or more, representative substrates in order to distinguish characteristics unique to one substrate activity or another.

P-300 Gel Filtration—In order to determine the approximate size of the enzyme, it was chromatographed on the polyacrylamide gel, P-300, and the elution profile is shown in Fig. 3. In this experiment, the fractions were assayed with two substrates, crotonyl-ACP (\( \text{trans-2 4-carbon-ACP} \)) and \( \text{cis-5-trans-2-dodecadienoyl-ACP} \). The activity peaks were found to be perfectly coincident, with the ratio of the two activities constant throughout the active fractions. The activity recovered in the pooled peak represented 80% of that applied to the column. In another experiment, the activity for \( \text{trans-2-dodecenoyl-ACP} \) was also found to cochromatograph with these activities. The elution position of catalase and hexokinase from the column are also indicated in Fig. 3. Comparison with these proteins allows an estimation of 170,000 for the apparent molecular weight of the dehydrase.

Polyacrylamide Gel Electrophoresis—When the highly purified dehydrase preparation was subjected to standard gel electrophoresis in 7.5% polyacrylamide gel in 7.5% polyacrylamide (19), much of the protein precipitated onto the top of the separating gel. The majority of the protein which did enter the gel ran as a diffuse band with an RF of about 0.26 (Fig. 4A). When the sample was prepared by dialysis in 8 M urea and dithiothreitol, as described in the legend of Fig. 4, and 8 M urea was included in the gel (20), less protein remained at the top of the gel after electrophoresis and the diffuse band stained more intensely, indicating that more of the protein had migrated to this position (Fig. 4B). A duplicate of this urea gel was sliced without staining, and the protein was eluted from the slices and dialyzed against buffer as described under “Experimental Procedure.” The dehydrase activity was found only in two 2-mm slices which coincided with the major diffuse band of the stained gel at RF 0.26. Recovery of activity from the gel was only about 20%. However, this eluted enzyme preparation was active with both crotonyl-ACP and \( \text{cis-5-trans-2-dodecadienoyl-ACP} \) and the ratio of activities with these two substrates was similar to the ratio in the sample applied to the gel.

pH Optima—Reaction rates for the highly purified enzyme preparation were tested at various pH values. For measuring the forward reaction, \( \text{cis-5-β-hydroxydodecenoyl-ACP} \) was used as the substrate. \( \text{cis-5-trans-2-Dodecadienoyl-ACP} \) was the substrate used for measurements of the hydration reaction. The results are plotted in Fig. 5. It is apparent that the rate-limiting step in both directions showed maximal activity at a pH of approximately 8.0.

Heat Inactivation—The dehydrase proved to be quite resistant...
FIG. 4. Polyacrylamide gel electrophoresis of β-hydroxyacyl-ACP dehydrase. Gels were prepared and run as described under "Experimental Procedure." The enzyme used in all cases had a specific activity of 15 μmoles per min per mg. Black threads mark the dye front. A, 32 μg of enzyme, 7.5% acrylamide, pH 8.8. B, 32 μg of enzyme, 7.5% acrylamide, pH 8.8, containing 8 M urea. Samples were dialyzed versus 8 M urea, 0.01 M dithiothreitol, and upper buffer for 3 hours at room temperature.

to heat inactivation in the buffer described in the legend of Fig. 5. An experiment is shown in Fig. 6 in which the enzyme was incubated at 80° for increasing lengths of time, quickly cooled, and the activity tested with two substrates. The first order plot of enzyme inactivation shows that the activities with both crotonyl-ACP and cis-5-trans-2-dodecadienoyl-ACP declined at identical rates. It should be noted that after 30 min at 80° almost 50% of the activity remained in both cases.

In other buffers the enzyme was found to be less stable. In 0.1 M Tris-HCl, pH 7.4, containing 10 mM dithiothreitol, the activity was destroyed after heating at 50° for 10 min, and in 0.1 M triethanolamine-HCl, pH 7.4, containing 10 mM dithiothreitol and 1 mM EDTA, the activity decreased to 40% in 10 min. At 50° in 0.1 M potassium phosphate, pH 7.2, on the other hand, the activity dropped only to 85% in 10 min.

Sensitivity to N-Ethylmaleimide—Early in the studies of this enzyme, maintenance of maximum activity was found to require the presence of high levels of reducing agent. Dithiothreitol, 5 to 10 mM, was therefore used routinely in preparation and storage of the enzyme, along with 1.0 mM EDTA to retard oxidation. In light of these observations, it was not surprising to find that the dehydrase was sensitive to inactivation by N-ethylmaleimide (NEM). As indicated in Fig. 7 enzyme inhibition increased with increasing concentrations of N-ethylmaleimide; at about 0.1 mM N-ethylmaleimide the enzyme was 50% inhibited. In addition, it should be noted that the decrease in enzyme activity at all concentrations of inhibitor tested were similar with each of three substrates: crotonyl-ACP, cis-5-trans-2-dodecadienoyl-ACP, and trans-2-hexadecenoyl-ACP.

In all of these studies of the β-hydroxyacyl-ACP dehydrase, the activities of the preparation for the substrates tested were seen to behave quite similarly. This strongly suggested the presence of a single protein having a substrate specificity which includes crotonyl-ACP, cis-5-trans-2-dodecadienoyl-ACP, and trans-2-hexadecenoyl-ACP. Thus the enzyme is active with (a) a short chain precursor of both saturated and unsaturated fatty acids, (b) a medium chain precursor of saturated fatty acids, (c) a medium chain precursor of unsaturated fatty acids, and (d) a long chain precursor of saturated fatty acids.

Purification of Dehydrase from Wild Type E. coli B—It was felt that if the entire purification procedure were carried out, obtaining at each step the ratio of activities for crotonyl-ACP and cis-5-trans-2-dodecadienoyl-ACP, conclusive evidence could be obtained as to whether single or multiple enzymes were responsible for the broad substrate specificity of the dehydrase preparation. In addition, purification of the enzyme from an extract of a wild type E. coli, strain B, would exclude the possibility that a peculiar effect of the mutation or of the mutagenesis used to obtain the K-12 mutant, L010, was responsible for some of the results obtained with the β-hydroxyacyl-ACP dehydrase purified from the mutant.
The procedure followed, shown in Table II, was that used previously (compare Table I), and a 500-fold purification was achieved. However, instead of beginning the purification with crude extract, the starting material was the supernatant of a 45% saturated ammonium sulfate solution of the crude extract. Thus the specific activity of this material was about 5 times that of the usual crude extract. However, the specific activity achieved was 12 µmoles per min per mg which is almost identical with that obtained previously with enzyme from extracts of E. coli. As shown in Table II, the ratio of activities with crotonyl-ACP and cis-5-trans-2-dodecadienoyl-ACP remained virtually constant at about 1.8 throughout the 500-fold purification.

When the kinetic constants for crotonyl-ACP, trans-2-dec-enoyl-ACP, and cis-5-trans-2-dodecadienoyl-ACP were determined with this enzyme preparation, the relationship of the $V_{\text{max}}$ values was similar to that seen with the purified K-12 enzyme. The maximal velocity with crotonyl-ACP was greatest, that with cis-5-trans-2-dodecadienoyl-ACP was 56% of the crotonyl-ACP value, and the maximal activity with trans-2-dec-enoyl-ACP was only 2 to 5% that with crotonyl-ACP.

**Relationship between β-Hydroxyacyl-ACP Dehydrase and Previously Reported Dehydrases**—The methods of preparation of the dehydrases of Majerus et al. (1) and of Mizugaki et al. (3) and those reported here were not dissimilar enough to exclude the possibility that the same protein had been isolated in each case. The former workers tested only 4-carbon substrates with their...
purified dehydrase. The latter group tested 4-, 6-, 8-, and 10-
carbon substrates with the "3-hydroxybutyryl-ACP dehydrase." The
relationships among the activities of these substrates bore a
striking similarity to those found with the 3-hydroxyacyl-ACP
dehydrase reported in this paper.

To answer the question of whether the dehydrases active with
3-hydroxyacyl-ACP compounds were distinct, a purification was
carried out according to the method of Mizugaki et al. (3), and the
ratio of the activities with crotonyl-ACP and cis-5-trans-2-
dodecadienoyl-ACP (4:1 to 12:2) was determined at each step.
Although the procedure which had provided the original workers
with a 500-fold purification of 3-hydroxybutyryl-ACP dehy-
drase was followed, the final enzyme preparation in this case was
only 70-fold increased in specific activity over the crude extract.
The 4:1 to 12:2 activity ratio was, however, constant through-
out the procedure. There was difficulty in keeping the activity
in solution in 0.01 M potassium phosphate buffer, and conse-
quently much enzyme was lost in steps in which the enzyme
was manipulated for any length of time in this buffer in the ab-


dence of other salts. As has been pointed out, this was also a
characteristic of the 3-hydroxyacyl-ACP dehydrase purified by
the procedure of Table I.

The peculiar elution pattern of the activity chromatographed
on Sephadex G-100 columns developed with 0.01 M phosphate
buffer, reported for the 3-hydroxybutyryl-ACP dehydrase (3),
was also seen in this preparation. The majority of the activity
was eluted with the void volume, while a smaller peak of activity
was eluted with the salt fraction, phenomena which were repro-
duced several times. In addition, when the large molecular
weight peak was pooled and rechromatographed in 0.01 M phos-
phate buffer on the same G-100 column, an identical pattern
of two peaks was seen. The enzyme in both peaks had the same
ratio of activities with the two test substrates. Neither the re-
relationship between the two peaks nor between these and the
single peak, which was eluted from G-100 near the void volume
when 0.1 M phosphate was used in the chromatography, was
studied further. However, in view of the observed insolubility
of the dehydrase in 0.01 M potassium phosphate buffer, it is sug-
gested that the latter peak eluted from Sephadex G-100 in this
buffer has no relevance to the molecular weight of the native
protein. As reported by Alberts et al. (23) with respect to the
transcarboxylase component of the aeryl CoA carboxylase
system of E. coli, highly aggregated or precipitated proteins may
be retarded on Sephadex columns, resulting in their elution near
the salt front.

**DISCUSSION**

Evidence available at the beginning of these studies indicated
the possibility that a number of enzymes, catalyzing the dehy-
drlation of various 3-hydroxyacyl-ACP intermediates, were
components of the E. coli fatty acid synthetase. It was clear that
the 3-hydroxydecanoyl thioester dehydrase was specific in utilizing
3-hydroxydecanoyl-ACP to produce the cis unsaturated
precursors of long chain unsaturated fatty acids (24), and that
lack of this enzyme had no detrimental effect on saturated fatty
acid synthesis (12). Dehydrases producing trans-2-unaturated
acyl-ACP intermediates, which were active with 4-carbon sub-
strates, had been purified by Majerus et al. (1) and by Mizugaki
et al. (3). The latter workers demonstrated that their enzyme
preparation was active with trans-2-acyl-ACP substrates of 4, 6,
and 8 carbon atoms, that the activity decreased with increasing
chain lengths, and that very little activity could be detected with
10-carbon substrates. With this background, the purification
of a dehydrase active with cis 5 trans 2 dodecadienoyl-ACP, an
intermediate in unsaturated fatty acid biosynthesis, was under-
taken. The first objective of the enzyme purification was to
determine whether the activity would prove to be specific for
unsaturated fatty acid precursors. If it were not, however, the
effort was expected to yield the dehydrase showing specificity
for both saturated and unsaturated intermediates of medium,
and perhaps longer, chain length. To our surprise, however,
the purified 3-hydroxyacyl-ACP dehydrase showed a broad range
of substrate activities, encompassing saturated fatty acid pre-
cursors from 4 to 16 carbon atoms in length and the 12-carbon
unsaturated intermediate. It had markedly reduced activity
with the 10-carbon substrate (Fig. 2). Despite the bimodal
nature of the dehydrase specificity profile with substrates of from
4 to 16 carbon atoms, it seems quite certain that a single enzyme
is responsible for these activities. This conclusion is based upon
the failure to separate the activities for crotonyl-ACP and cis-5-
trans-2-dodecadienoyl-ACP by disc gel electrophoresis or gel
filtration, by the copurification of these activities from extracts
of E. coli B, and by a comparison of the properties of the enzy-
matic activities catalyzing reactions with these two substrates
and, in those experiments in which it was tested, with trans-2-
hexadecenoyl-ACP. The bimodal substrate activity profile
cannot be explained at this time. The simplest model allowing
for the relative inactivity of the 10-carbon substrate would in-
volve two distinct binding sites. One would be relatively specific

\[
\begin{align*}
V_{\text{max}} & = 3.6 \times 10^{-8} \\
K_m & = 2.4 \times 10^{-8} \\
4 & \text{ chain length}
\end{align*}
\]

**Fig. 8.** Comparison of properties of 3-hydroxydecanoyl thio-
ester dehydrase and 3-hydroxyacyl-ACP dehydrase. Data on
3-hydroxyacyl-ACP dehydrase (Column A) are either contained
in this paper or in unpublished results of C. H. Birge. \(K_m\) of
3-hydroxydecanoyl-ACP for 3-hydroxyacyl-ACP thioester dehy-
drase (Column B) was taken from Reference 2. Other data on
this dehydrase were derived from References 7 and 8. The rela-
tive rates shown in the figure refer to maximal velocities of dehy-
dration with the indicated chain length thioesters. Solid bars
represent 3-hydroxydecanoyl thioester dehydrase; cross-hatched
bars, 3-hydroxyacyl-ACP dehydrase.
for substrates 4 to 8 carbon atoms in length, the second for those 13 to 18 carbon atoms long.

The dehydrase described here, \( \beta \)-hydroxyacyl-ACP dehydrase, and in the report of Birge et al. (2), is considered to be the same enzyme as that partially purified by Majerus et al. from a K-12 strain (1). It also has pronounced similarities to the dehydrase purified by Mizugaki et al. (3). However, since they did not test the enzyme with longer substrates, these workers assumed that it was specifically a short chain dehydrase, referring to it as \( \beta \)-hydroxybutyryl-ACP dehydrase. Other properties which the two enzymes have in common include their relative stability upon incubation at higher temperatures, their strong affinity for gels of the calcium phosphate type, their specificity for acyl-ACP's as opposed to CoA thioesters, and their sensitivity to sulphydryl reagents.

Subsequent to the report of the purification of \( \beta\)-hydroxybutyryl-ACP dehydrase (3), Mizugaki et al. reported the partial purification of two other dehydrases: (a) a medium chain dehydrase, most active with an 8-carbon acyl-ACP substrate, and (b) a long chain dehydrase, most active with a 16-carbon acyl-ACP substrate (4). Neither enzyme preparation was highly purified, and the former was contaminated with substantial amounts of \( \beta\)-hydroxydecanoyl thioester dehydrase. Since the broad substrate specificity of the \( \beta\)-hydroxyacyl-ACP dehydrase shown in the present work could account for all the dehydration reactions producing \( \alpha\)-unsaturated acyl-ACP intermediates of fatty acid synthesis, the presence of other dehydrases in the cell might appear to be redundant. On the other hand, overlapping substrate specificities with relation to chain length has been shown among the enzymes involved in fatty acid metabolism (25). Thus the interpretation of the specific function of various dehydrases in vivo must await further information derived from highly purified enzyme preparations or perhaps the isolation of mutants lacking each of the individual dehydrases.

The co-ordination of the activity of the \( \beta\)-hydroxyacyl-ACP dehydrase with that of \( \beta\)-hydroxydecanoyl thioester dehydrase may be an important factor, perhaps the most important factor, in determining the relative quantities of saturated and unsaturated fatty acids synthesized by the cell. From information collected in this laboratory and from published data (2, 7, 8), a comparison of the properties of the two dehydrases has been made with respect to their abilities to compete for the common substrate, \( \beta\)-hydroxydecanoyl-ACP. The data are summarized in Fig. 8 where it is seen that estimates of the quantities of the enzymes per g of crude protein and the \( K_m \) values for the substrate are extremely similar. A comparison of the estimated maximal velocities is more difficult since it involves rates obtained with ACP substrates made in two different laboratories and, as mentioned above, it is possible that the quality of these substrates varies when different synthetic procedures are utilized. What should be noted, however, besides the fact that the maximal velocities at the 10-carbon level appear to be fairly similar, is the complementary pattern of the rates for 8-, 10-, and 12-carbon substrates. It seems plausible to attribute significance to the rate profiles, supported as they are by the other data of Fig. 8. That is to say, if the higher rates exhibited by the \( \beta\)-hydroxyacyl-ACP dehydrase with other substrates, such as those of chain lengths 8 and 12, were to prevail also at the 10-carbon level, the introduction of a cis double bond by \( \beta\)-hydroxy-

decanoyl thioester dehydrase would be a rare event. Instead, the yield of saturated and unsaturated fatty acids in vivo is actually about equal, and slanted toward the production of unsaturated fatty acids in vitro.

The phospholipids of E. coli, the major lipid component of these cells, are found to contain more saturated fatty acids when the cells are grown at higher temperatures. This could be explained, in part, by the finding of Sinensky (26) that the activities of \( \alpha\)-glycerol 3-phosphate and monooeyl \( \alpha\)-glycerol 3-phosphate acyltransferases, two early enzymes in phospholipid synthesis, respond to temperature variation in a way that indicates they may be responsible for the changes seen in fatty acid composition of phospholipids of cells grown at different temperatures. The availability of the purified \( \beta\)-hydroxyacyl-ACP dehydrase now permits experimentation to determine whether this enzyme also responds to temperature changes or other possible effectors in a way that might explain the variation in the ratio of unsaturated to saturated fatty acids found in the cell under different growth conditions.

REFERENCES

18. MUNKRES, K. D., AND RICHARDS, F. M. (1965) Arch. Biochem. Biophys. 109, 466
Acyl Carrier Protein: XVII. PURIFICATION AND PROPERTIES OF β-
HYDROXYACYL ACYL CARRIER PROTEIN DEHYDRASE
Claire H. Birge and P. Roy Vagelos


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