Gould, N. R., & Liener, I. E. (1965) *Biochemistry 4*, 90–98.
Hayaski, R., Bai, Y., & Hata, T. (1975) *J. Biochem. (Tokyo) 77*, 1313.
Jackson, I. M., & Reichlin, S. (1974) *Life Sci. 14*, 2259–2266.
Kenny, A. J., Booth, A. G., George, S. G., Ingram, J., Kershaw, P., Wood, E., & Young, A. R. (1976) *Biochem. J. 155*, 169–182.
Knisatchek, H., Kleinkauf, H., & Bauer, K. (1980) *FEBS Lett. 111*, 157–161.
Kraut, J. (1977) *Annu. Rev. Biochem. 46*, 331–358.
Means, G. E., & Feeney, R. E. (1971) in *Chemical Modification of Proteins*, pp 105–138, Holden-Day, San Francisco, CA.
Means, G. E., & Wu, H.-L. (1979) *Arch. Biochem. Biophys. 194*, 526–530.
Mounter, L. A., Alexander, H. L., Tuck, K. D., & Dien, L. T. H. (1956) *J. Biol. Chem. 226*, 867–872.
Murachi, T., & Yasui, M. (1965) *Biochemistry 4*, 2275–2282.
Murachi, T., Miyake, T., & Yamasaki, N. (1970) *J. Biochem. (Tokyo) 68*, 239–244.
Oliver, C., Eskay, R. L., Ben-Jonathan, N., & Porter, J. C. (1974) *Endocrinology 95*, 540–546.
Orlowski, M., Wilk, E., Pearce, S., & Wilk, S. (1979) *J. Neurochem. 33*, 461–469.
Peller, L., & Alberty, R. A. (1959) *J. Am. Chem. Soc. 81*, 5907–5914.
Powers, J. C. (1978) *Chem. Biochem. Amino Acids, Pept., Proteins 4*, 100–103.
Rupnow, J. H., Taylor, W. L., & Dixon, J. E. (1979) *Biochemistry 18*, 1206–1212.
Shaw, E. (1970) *Enzymes, 3rd Ed. 1*, 91–146.
Tate, S. S. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1780 (abstr.).
Taylor, W. L., & Dixon, J. E. (1980) *Biochem. Biophys. Res. Commun. 94*, 9–15.
Taylor, W. L., Andrews, P. C., Henrickson, C., & Dixon, J. E. (1980) *Anal. Biochem. 105*, 58–64.
Vallee, B. L., & Riordan, J. F. (1969) *Annu. Rev. Biochem. 38*, 733–794.
Whitaker, J. R. (1969) *Biochemistry 8*, 1896–1901.
Whitaker, J. R., & Perez-Villasenor, J. (1968) *Arch. Biochem. Biophys. 124*, 70–78.
Yoshimoto, T., Orlowski, R. L., & Walter, R. (1977) *Biochemistry 16*, 2942–2948.

An Essential Methionine in Pig Kidney General Acyl-CoA Dehydrogenase†

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ABSTRACT: The flavoprotein pig kidney general acyl-CoA dehydrogenase contains a single catalytically essential methionine residue/FAD which reacts with iodoacetate at pH 6.6. S-Carboxymethylation of this residue generates an inactive enzyme derivative which retains FAD and the tetrameric structure of the native protein. The derivative binds octanoyl-CoA and palmitoyl-CoA with concomitant perturbation of the flavin chromophore, but the characteristic spectrum of the reduced enzyme·enoyl-CoA complex is not observed. In addition, octanoyl-CoA strongly protects the native enzyme against alkylation with iodoacetate. These results suggest that the methionine residue is within the active center of acyl-CoA dehydrogenase. Carboxymethylation of this residue may disrupt the precise orientation of the substrate required to achieve transfer of reducing equivalents to the flavin. Pig kidney general acyl-CoA dehydrogenase does not contain exposed catalytically essential cysteine residues.

Mammalian acyl-CoA dehydrogenases comprise an important group of closely related flavoproteins which catalyze the insertion of a trans-2,3 double bond in their acyl thioester substrates during β-oxidation. Three classes of dehydrogenases have been isolated from pig liver with overlapping substrate specificities for short (Green et al., 1954), medium (Crane et al., 1956; Hall & Kamin, 1975), and long (Hauge et al., 1956; Hall et al., 1976) chain fatty acyl-CoA thioesters. These dehydrogenases transfer the reducing equivalents generated in this oxidation to a second flavoprotein, electron-transferring flavoprotein (ETF;[1] Crane & Beinert, 1956; Hall et al., 1979), which interacts with the respiratory chain probably at the level of a membrane-bound iron–sulfur flavoprotein (Ruzicka & Beinert, 1977).

We have previously reported the purification and properties of an acyl-CoA dehydrogenase of general specificity from pig kidney (Thorpe et al., 1979). The present study describes the carboxymethylation of an essential methionine in this flavoprotein and some of the properties of the derivatized enzyme.

Experimental Procedures

Materials. Pig kidney general acyl-CoA dehydrogenase was purified as described previously (Thorpe et al., 1979; Thorpe, 1980). All enzyme concentrations refer to bound FAD with the experimentally determined ϵ_{446} value of 15.4 mM^{-1} cm^{-1} (Thorpe et al., 1979). Visible and UV spectra were recorded on a Cary 219 instrument. All buffers contained 0.3 mM EDTA. Where appropriate, iodo[1-^{14}C]acetic acid (Amersham) was diluted to a specific activity of 0.55 μCi/μmol with unlabeled iodoacetic acid from Sigma. Solutions of iodoacetic acid were kept dark. Acyl-CoA thioesters were from P-L Biochemicals, and all other reagents were of analytical grade from commercial sources.

Enzyme Assays. Acyl-CoA dehydrogenase was assayed with octanoyl-CoA by using phenazine methosulfate and dichlorophenolindophenol as described previously (Thorpe et al.,

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[1] Abbreviations used: ETF, electron-transferring flavoprotein; SCM, *S*-carboxymethyl; CoA, coenzyme A.

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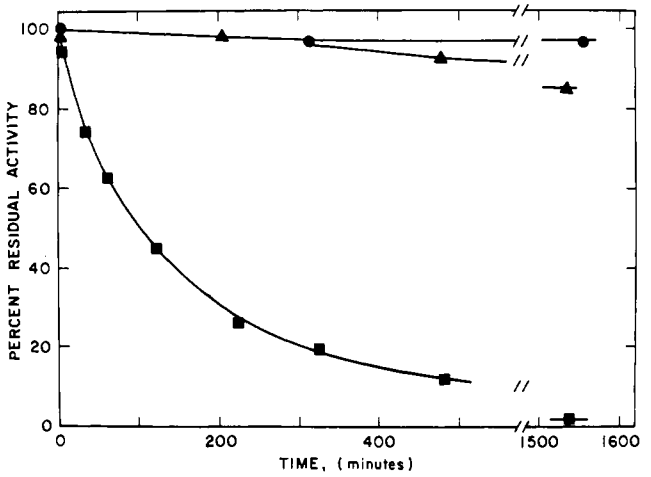

FIGURE 1: Inactivation of pig kidney general acyl-CoA dehydrogenase by iodoacetic acid. The control sample (●) contained 2.88 μM enzyme flavin in 0.8 mL of 100 mM phosphate buffer, pH 6.6. A second sample (■) contained, in addition, 30 mM iodoacetic acid adjusted to the same pH, and a third tube (▲) contained 30 mM iodoacetic acid plus 120 μM octanoyl-CoA. Incubations were started by the addition of enzyme, and 5-μL aliquots were withdrawn and diluted directly into assay cuvettes. Activities are expressed as percentages of the control sample at zero time.

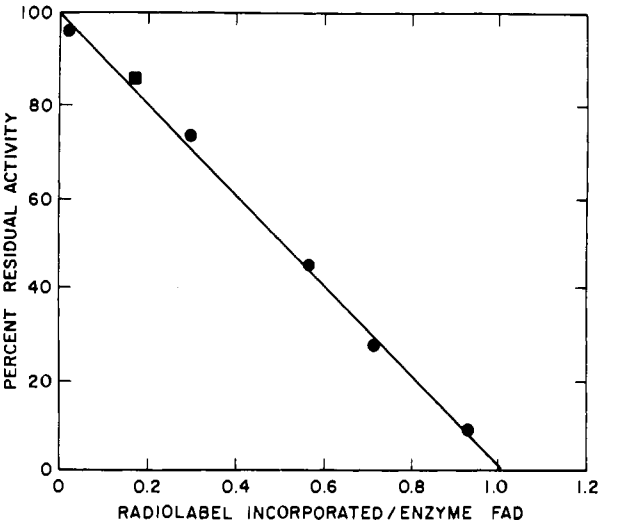

FIGURE 2: Percentage residual activity vs. extent of alkylation of acyl-CoA dehydrogenase by iodoacetic acid. The incubation contained 50 μM enzyme flavin and 30 mM iodo[1-^{14}C]acetic acid in 0.5 mL of 100 mM phosphate buffer, pH 6.4, at 25 °C. At intervals, 40-μL aliquots were withdrawn and applied to a 5 × 185 mm Sephadex G-25 column equilibrated with 100 mM phosphate, pH 6.4, and gel filtered in subdued light at a flow rate of 6 mL/h. No significant loss of flavin is incurred during gel filtration. Fractions were collected every 2 min directly into scintillation vials for counting. Immediately after gel filtration was initiated, a further 5-μL sample was diluted with 95 μL of 100 mM phosphate, pH 6.4, 0 °C, and rapidly assayed. This procedure was repeated at 2, 50, 130, 237, and 540 min to obtain the points shown in the figure. The experiment was repeated including 150 μM octanoyl-CoA. The incorporation obtained at 540 min in the presence of octanoyl-CoA is shown by the square symbol.

1979), except that the phosphate buffer concentration was raised from 20 to 50 mM. Samples of the enzyme incubation mixtures could be assayed directly without removal of iodoacetate or octanoyl-CoA since neither component influenced the rate of a standard assay under the conditions employed here.

Radioactivity Measurements. Samples were counted in a Beckman LS-100 instrument by using 10 mL of liquid scintillation cocktail (ACS, Amersham). Counting efficiency was determined by using internal standards.

Amino Acid Analysis. Samples were dissolved in 6 M HCl and deoxygenated by cycles of evacuation and flushing with nitrogen combined with freezing and thawing. Tubes were sealed under vacuum and heated for 22 h at 110 °C. The hydrolysates were evaporated while vortexing took place, and the vapors were condensed in a dry ice/acetone trap to determine the loss of volatile radioactive material (Goren et al., 1968). Analyses were run on a Beckman 119C amino acid analyzer with a 3-h program. Effluent from the colorimeter was collected in 1.5-min fractions and transferred to scintillation vials. After the peak positions were noted, three drops of concentrated HCl were added to each vial to discharge the blue color prior to counting. *S*-([1-^{14}C]Carboxymethyl)-methionine was prepared as described earlier (Gundlach et al., 1959).

Gel Filtrations. Samples of native and iodoacetate-modified enzyme were gel filtered (at 5 mL/h) on a 1 × 49 cm Sephacryl-S200 column (Pharmacia) equilibrated with 100 mM phosphate buffer, pH 7.6 (4 °C), to compare their apparent molecular weights. The molecular weight of the native enzyme had been previously estimated by this method (Thorpe et al., 1979). Polyacrylamide gel electrophoresis was performed as described earlier (Thorpe et al., 1979).

Anaerobic Titration. Anaerobic titrations of the enzyme derivative were conducted as described by Williams et al. (1979) except that 1-mL capacity semimicro anaerobic cuvettes were used.

Results

Figure 1 shows the inactivation of pig kidney general acyl-CoA dehydrogenase by 30 mM iodoacetic acid in 100 mM phosphate buffer, pH 6.6, at 25 °C. Loss of activity is half-complete in 100 min, and after 25 h approximately 2% of the original activity remains. Strong protection against this inactivation is afforded by 120 μM octanoyl-CoA (Figure 1). A control sample, incubated in the absence of iodoacetic acid and substrate, loses very little activity during the experiment.

When alkylations are performed with 2.9 μM enzyme FAD (Figure 1), semilogarithmic plots of residual activity vs. time appear linear over two half-lives and then show a slight upward curvature. This deviation from linearity is much less pronounced at higher enzyme levels; for example, at 20 μM enzyme, inactivation follows first-order kinetics over at least three half-lives. In Figure 2, the inactivation was repeated by using iodo[1-^{14}C]acetic acid with more concentrated enzyme. Aliquots were withdrawn at intervals, and the activity remaining and incorporation of radiolabel were correlated. Extrapolation of the points to zero activity indicates that inactivation is associated with the modification of 1.0 residues/FAD. Inactivation does not lead to release of significant amounts of flavin (see legend to Figure 2).

The experiment was then repeated in the presence of 150 μM octanoyl-CoA. As expected, incorporation of radiolabel was much slower; 0.18 label/FAD with 85% residual activity was observed at 9 h compared to 0.93 label/FAD with 9% remaining activity in the unprotected sample over the same time period. Enzyme activity is not regained nor is radiolabel lost on prolonged dialysis against phosphate buffer. Gel filtration and polyacrylamide gel electrophoresis studies indicate that the derivative retains the tetrameric structure of the native flavoprotein (see Experimental Procedures).

A methionine residue was identified as the site of alkylation by amino acid analysis of the radiolabeled enzyme derivative by monitoring the column effluent for radioactivity (Figure 3). SCM-Met decomposes during acid hydrolysis to a mixture

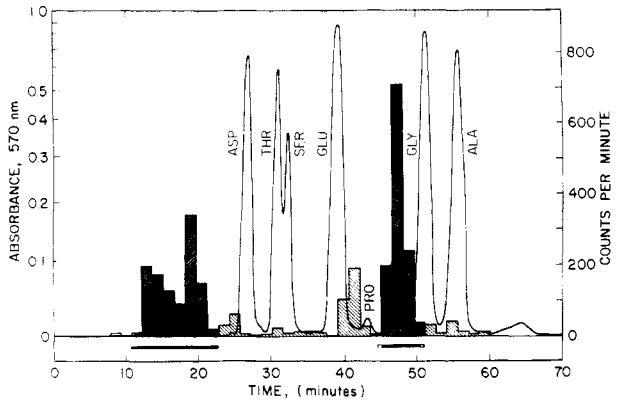

FIGURE 3: Amino acid analysis of the modified enzyme. The derivative (1.2 radiolabel/FAD) prepared as described in the legend to Figure 2 was hydrolyzed, and the effluent from the analyzer was collected for radioactive counting. The hatched areas represent radioactivity. The dense hatched areas correspond to *S*-([1-^{14}C]carboxymethyl)-methionine breakdown products and were assigned by comparison with the radioactive profile (solid bars indicate positions of the peaks) obtained by using an authentic sample of the methionine derivative (see Experimental Procedures).

Table I: Effect of Sulfhydryl Reagents on General Acyl-CoA Dehydrogenase, 25 °C

treatment	residual act.[a] (%)
iodoacetate (30 mM, pH 6.6,[b] 100 min)	50
iodoacetamide (30 mM, pH 6.6, 100 min)	61
N-ethylmaleimide (3.7 mM, pH 7.6, 23 h)	100
phenylmercuric acetate (0.26 mM, pH 7.6, 3 h)	98
$(Nbs)_2$[c] (1 mM, pH 7.6, 4 h)	88

[a] Expressed as percentage of control. [b] All buffers were 100 mM phosphate and the enzyme concentrations used were 2.6–2.9 μM. [c] $(Nbs)_2$, 5,5′-dithiobis(2-nitrobenzoic acid).

of ninhydrin-positive and ninhydrin-negative products whose composition depends on the exact hydrolysis conditions used (Goren et al., 1968; Gundlach et al., 1959; bold hatched areas in Figure 3). The SCM-Met content was calculated as described by Goren et al. (1968), summing the radioactivity under SCM-Met breakdown products, and correcting for a loss of volatile material during sample preparation (see Experimental Procedures). The enzyme derivative analyzed in Figure 3 contained 1.2 radiolabels/FAD of which 1.08 were attributable to SCM-Met. The major ninhydrin-positive breakdown product, SCM-Hcy (Gundlach et al., 1959), is clearly visible between proline and glycine. The small radioactive peak (0.02 label) just before aspartic acid is probably SCM-Cys, and the peak between glutamic acid and proline (0.1 label) corresponds to the position expected for 1-CM-His (Goren et al., 1968). No further radioactive peaks appear after alanine, ruling out the presence of appreciable levels of 3-CM-His and ε-CM-Lys in the hydrolysate (Goren et al., 1968). As would be expected for alkylation of a methionine side chain, inactivation is relatively pH independent. The pseudo-first-order rate constant for inactivation with 30 mM iodoacetate increases by only 10% between pH 6.3 and 7.6 (results not shown).

In view of the low yield of SCM-Cys observed in Figure 3, several other potential sulfhydryl reagents were tested for their effect on enzyme activity. Pig kidney general acyl-CoA dehydrogenase contains 5–6 cysteine residues/FAD which react with 5,5′-dithiobis(2-nitrobenzoic acid) under denaturing conditions, although none are readily reactive in the native enzyme (Thorpe et al., 1979). The data shown in Table I

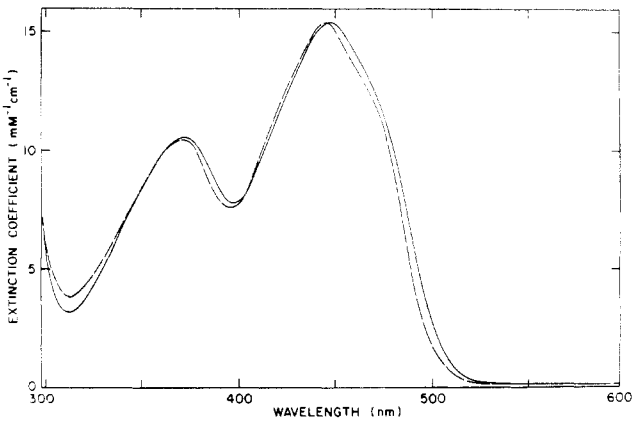

FIGURE 4: Comparison of the visible spectra of native and alkylated pig kidney general acyl-CoA dehydrogenase. Spectra were recorded in 100 mM phosphate buffer, pH 7.6, 25 °C; (—) native enzyme, (- - -) modified enzyme.

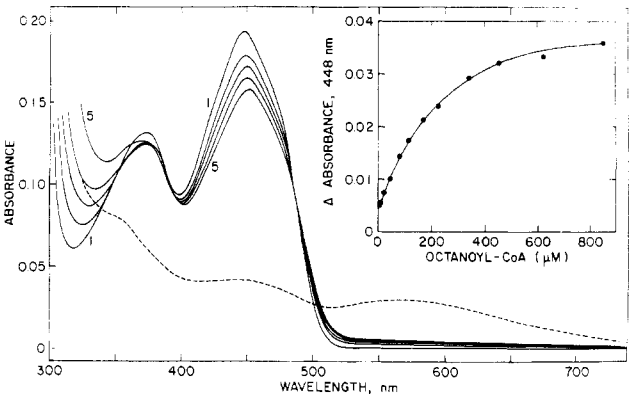

FIGURE 5: Effect of octanoyl-CoA on the visible spectrum of the alkylated enzyme. Curve 1: 12.3 μM alkylated enzyme in 0.7 mL of 100 mM phosphate buffer, pH 7.6, at 25 °C. Curves 2–5: With the addition of 80, 170, 340, and 860 μM octanoyl-CoA. Intermediate spectra in the titration (see inset) have been omitted for clarity. No further spectral changes occurred after each addition of substrate. The spectra have been corrected for dilution. The dashed line is the spectrum of 12.3 μM native enzyme in the presence of 30 μM octanoyl-CoA.

suggest that the enzyme does not have exposed, rapidly reacting cysteine residues which are essential for catalytic activity.

The visible spectrum of the modified enzyme, when freed from excess iodoacetic acid by gel filtration, shows small but significant changes from that of the native enzyme (Figure 4). A slight shoulder appears at 470 nm, and λ_{max} for the derivatives is blue shifted by about 1 nm. The spectrum of the modified enzyme is unchanged on storage for 1 month at 4 °C, indicating that release of flavin must be slow under these conditions. The enzyme derivative, like the native protein, does not exhibit appreciable flavin fluorescence.

Figure 5 shows the effect of increasing concentrations of octanoyl-CoA on the spectrum of the modified flavoprotein and, for comparison, the spectrum of the native enzyme in the presence of this substrate. Clearly, the native and modified enzymes differ widely in behavior. In the native dehydrogenase, reduction of the flavin is accompanied by the appearance of a broad long-wavelength band at 570 nm (Crane et al., 1956; Thorpe et al., 1979; Hall et al., 1979) which has been attributed to a charge-transfer interaction between reduced flavin as the donor and *trans*-2-octenoyl-CoA as the acceptor (Massey & Ghisla, 1974; Murfin, 1974). Maximal formation of this complex occurs at approximately 1 equiv of substrate/FAD (Thorpe et al., 1979; Hall et al., 1979). In

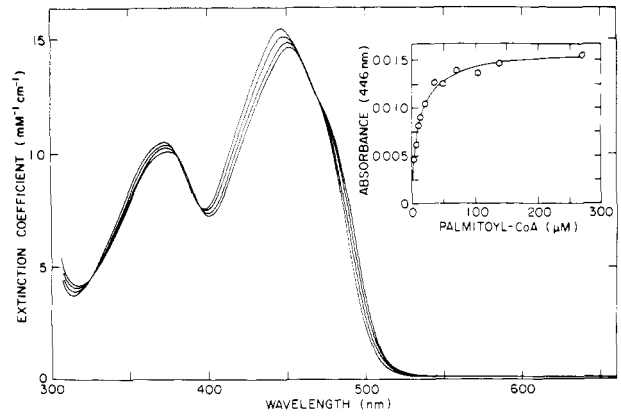

FIGURE 6: Titration of the modified enzyme with palmitoyl-CoA. Curve 1: 11.8 μM alkylated enzyme in 0.7 mL of 100 mM phosphate buffer, pH 7.6, at 25 °C. Curves 2–4: After the addition of 3.6, 21, and 50 μM palmitoyl-CoA. No further spectral changes occurred after each addition of substrate. Intermediate spectra have been omitted for clarity. Data in the inset have been corrected for dilution.

contrast, the spectral change observed for the derivative requires a large excess of substrate for completion (K_D for octanoyl-CoA = 170 μM under these conditions; see inset, Figure 5). Associated with a small absorbance decrease at 448 nm is a weak long-wavelength tail extending to 750 nm. The appearance of this band correlates with the decrease at 448 nm and cannot be due to the 5% residual native enzyme which is titrated in the first octanoyl-CoA addition. In addition, the isobestic points obtained during octanoyl-CoA titrations of the derivative and native enzymes are clearly different, at 486 (Figure 5) and 499 nm (Thorpe et al., 1979), respectively. The changes shown in Figure 5 were completed before spectra could be recorded, and no further spectral changes were observed between each substrate addition. Spectral changes analogous to those observed in Figure 5 were observed when oxidized enzyme 12.9 μM in phosphate buffer, pH 7.6, was mixed anaerobically with 419 μM octanoyl-CoA.

The experiment shown in Figure 5 indicates that the characteristic reduced enzyme·octenoyl-CoA charge-transfer interaction is not observed during titration of the oxidized modified enzyme with octanoyl-CoA. It was therefore of interest to examine whether the reduced carboxymethylated enzyme could form long-wavelength bands with octenoyl-CoA. The dihydroflavin form of the enzyme was prepared by dithionite titration (see Experimental Procedures), and, as observed with the native enzyme (Thorpe et al., 1979), appreciable levels of the blue neutral semiquinone were observed at the midpoint of the titration. After full reduction, the enzyme (11.8 μM) was mixed anaerobically with 228 μM octenoyl-CoA. No long-wavelength band appears over a period of several hours, in marked contrast to the broad band exhibited by the native enzyme (ϵ_{560} = 2.5 mM^{-1} cm^{-1}; Thorpe et al., 1979). Similarly, no significant perturbation of the dihydroflavin spectrum was observed. Introduction of air to the anaerobic cuvette led to the rapid reoxidation of the enzyme to regenerate the original flavoprotein spectrum.

Figure 6 shows a spectrophotometric titration of the enzyme derivative with palmitoyl-CoA. This long-chain analogue effects a 6-nm red shift in λ_{max} with increased resolution of the shoulder at 470 nm, suggestive of a decrease in polarity of the flavin environment (Palmer & Massey, 1968). These changes are similar to those induced by palmitoyl-CoA in the native enzyme (Thorpe et al., 1979) except that partial reduction of the flavin with concomitant appearance of a long-wavelength band is not observed with the inactive enzyme derivative. Palmitoyl-CoA binds considerably more tightly (K_D = 12 μM; inset, Figure 6) than octanoyl-CoA to the modified enzyme.

Discussion

This work has identified an essential methionine residue in pig kidney general acyl-CoA dehydrogenase. Three lines of evidence suggest that this residue forms part of the active site of the enzyme. Carboxymethylation is accompanied by a significant perturbation of the visible spectrum of the enzyme. The modified protein still binds octanoyl- or palmitoyl-CoA, but subsequent reduction of the flavin with formation of the characteristic spectrum of the $EFlH_2$·enoyl-CoA complex does not occur. Finally, octanoyl-CoA provides strong protection against inactivation with iodoacetate.

Although the derivative does not form the characteristic $EFlH_2$·octenoyl-CoA spectrum, a weak long-wavelength band is observed in Figure 5. Turbidity of the sample has been ruled out as an explanation, since the isobestic point at 486 nm is retained throughout the titration, the absorbance at 800 nm is zero, and the spectrum is unaffected by centrifugation. Both *Megasphaera elsdenii* butyryl-CoA dehydrogenase (Engel & Massey, 1971) and the general acyl-CoA dehydrogenase from pig liver (McKean et al., 1979) form long-wavelength bands with concomitant decrease in absorbance at 450 nm in the presence of acetoacetyl-CoA. These spectral changes have been ascribed to the formation of charge-transfer complexes between the enolate form of the ligand as the donor and oxidized flavin as the acceptor (Massey & Ghisla, 1974; Engel & Massey, 1971). A recent resonance Raman study of the liver dehydrogenase supports this assignment (Benecky et al., 1979). The long-wavelength tail in Figure 5 could conceivably represent a similar complex between the enol or enolate form of octanoyl-CoA and the oxidized modified enzyme.

This study was instigated in an attempt to identify amino acid side chains which serve to activate octanoyl-CoA prior to the flavin reduction step. Cornforth (1959) suggested that an active-site base residue removes an α proton from the bound thioester moiety in the acyl-CoA dehydrogenase. More recent work with several other flavoproteins indicates that proton abstraction, with the formation of a substrate-derived carbanion, is indeed a likely early step in the reductive half-reactions of these enzymes (Walsh et al., 1971, 1973; Porter et al., 1973; Ghisla & Massey, 1977). Chemical modification of such a base residue might be expected to abolish flavin reduction but still permit substrate binding to the enzyme derivative. While the S-carboxymethylated enzyme studied here shows this behavior, it appears unlikely that methionine would play a primary role in the substrate activation envisaged above. A plausible alternative is that the methionine residue is involved in the maintenance of the active-site environment and that introduction of the polar *S*-carboxymethyl moiety disrupts the alignment of reactants within the active center, preventing subsequent reduction of the flavin.

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References

Benecky, M., Li, T. Y., Schmidt, J., Frerman, F., Watters, K. L., & McFarland, J. (1979) *Biochemistry 18*, 3471–3476.

Cornforth, J. W. (1959) *J. Lipid Res. 1*, 3–28.

Crane, F. L., & Beinert, H. (1956) *J. Biol. Chem. 218*, 717–731.
Crane, F. L., Mii, S., Hauge, J. G., Green, D. E., & Beinert, H. (1956) *J. Biol. Chem. 218*, 701–716.
Engel, P. C., & Massey, V. (1971) *Biochem. J. 125*, 889–902.
Ghisla, S., & Massey, V. (1977) *J. Biol. Chem. 252*, 6729–6735.
Goren, H. J., Glick, D. M., & Barnard, E. A. (1968) *Arch. Biochem. Biophys. 126*, 607–623.
Green, D. E., Mii, S., Mahler, H. R., & Bock, R. M. (1954) *J. Biol. Chem. 206*, 1–12.
Gundlach, H. G., Moore, S., & Stein, W. H. (1959) *J. Biol. Chem. 234*, 1761–1764.
Hall, C. L., & Kamin, H. (1975) *J. Biol. Chem. 250*, 3476–3486.
Hall, C. L., Heijkenskjöld, L., Bârtfai, T., Ernster, L., & Kamin, H. (1976) *Arch. Biochem. Biophys. 177*, 402–414.
Hall, C. L., Lambeth, J. D., & Kamin, H. (1979) *J. Biol. Chem. 254*, 2023–2031.
Hauge, J. G., Crane, F. L., & Beinert, H. (1956) *J. Biol. Chem. 219*, 727–733.
Massey, V., & Ghisla, S. (1974) *Ann. N.Y. Acad. Sci. 227*, 446–465.
McKean, M. C., Frerman, F. E., & Mielke, D. M. (1979) *J. Biol. Chem. 254*, 2730–2735.
Murfin, W. (1974) Ph.D. Dissertation, Washington University.
Palmer, G., & Massey, V. (1968) *Biol. Oxid.*, 263–300.
Porter, D. J. T., Voet, J. G., & Bright, H. J. (1973) *J. Biol. Chem. 248*, 4400–4416.
Ruzicka, F. J., & Beinert, H. (1977) *J. Biol. Chem. 252*, 8440–8445.
Thorpe, C. (1980) *Methods Enzymol.* (in press).
Thorpe, C., Matthews, R. G., & Williams, C. H. (1979) *Biochemistry 18*, 331–337.
Walsh, C. T., Schonbrunn, A., & Abeles, R. H. (1971) *J. Biol. Chem. 246*, 6855–6866.
Walsh, C. T., Lockridge, O., Massey, V., & Abeles, R. H. (1973) *J. Biol. Chem. 248*, 7049–7054.
Williams, C. H., Jr., Arscott, L. D., Matthews, R. G., Thorpe, C., & Wilkinson, K. D. (1979) *Methods Enzymol. 62*, 185–198.