Evidence That the Greening Ligand in Native Butyryl-CoA Dehydrogenase Is a CoA Persulfide*

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Yellow butyryl-CoA dehydrogenase and general acyl-CoA dehydrogenase are "greened" by a mixture of coenzyme A plus elemental sulfur. The resultant stable complex contains an identical ligand with that present in native green butyryl-CoA dehydrogenase and has the same broad absorption band centered at 710 nm. Evidence is presented that the greening ligand is a CoA persulfide, possibly a mimic of the substrate carbanion and most long wavelength absorption bands. Engel and Massey (7) proposed that a charge-transfer interaction between an unknown bound acyl-CoA and the protein-bound FAD is responsible for the 710 nm absorption band. However, attempts to identify the putative acyl group by gas-liquid chromatography and mass spectrometry were unsuccessful. In addition, the greening of native enzyme by some sulfhydryl reagents (3, 9), the abolition of greenness by dithionite (13), and the instability of the released "re-greening factor" (8), were puzzling and seemed inconsistent with a bound acyl-CoA species.

General acyl-CoA dehydrogenase (EC 1.3.99.3) when isolated from pig tissues is yellow (10-12) and contains no bound CoA compounds (12). On addition of acetoacetyl-CoA, however, it forms a complex with an absorption spectrum strikingly similar (13) to that formed by butyryl-CoA dehydrogenase with the same ligand (7). The experiments reported here show that this enzyme is also, like butyryl-CoA dehydrogenase, able to form a green complex with an absorption band maximal at 710 nm. The results strongly suggest that the greening ligand in both enzymes is a CoA persulfide, CoA-S-S-, and that the 710 nm band is due to a charge-transfer interaction between this ligand and the protein-bound FAD.

EXPERIMENTAL PROCEDURES

Enzymes—Pig kidney general acyl-CoA dehydrogenase was purified as previously described (14). The purification of butyryl-CoA dehydrogenase from Megasphaera elsdenii was performed according to Engel (15)—including the final Sephadex G-200 step. Yellow butyryl-CoA dehydrogenase was prepared from native enzyme by repeated anaerobic dithionite dialyses. Bound flavin concentrations were determined spectrophotometrically. The extinction coefficients used were as follows for general acyl-CoA dehydrogenase, 15.4 × 10^4 M^-1 cm^-1 at 446 nm (14); for yellow butyryl-CoA dehydrogenase, 12.5 × 10^4 M^-1 cm^-1 at 450 nm (3); and for green butyryl-CoA dehydrogenase, 10.4 × 10^4 M^-1 cm^-1 at 430 nm (15). Experiments were performed in 100 mM KPi, pH 7, at 25 °C unless otherwise stated. Spectra were recorded with a Cary 219 scanning spectrophotometer. The greenness of an enzyme sample is expressed either as a ratio between the absorbance at 710 nm and 430 nm, or as a percentage of the maximum greenness. The highest A710/A430 ratio theoretically obtainable has been estimated to be 0.54 (3) and hence a sample with A710/A430 = 0.27 is designated as 50% green.

Radioactive Labeling Studies—[Adenine-8-14C]CoASH was purchased from P-L Biochemicals. For experiments, the specific activity was diluted to 4.40 × 10^4 Bq nmol^-1. Na2S was purchased from the Radiochemical Centre, Amersham, and diluted with Na2S in nitrogen-

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saturated water. The specific activity of this solution was \(1.82 \times 10^5\) Bq nmol\(^{-1}\). Corrections for the decay of \(^{38}\)S were applied as recommended by the manufacturers. Both labeled solutions were stored at \(-20^\circ\)C. The sodium sulfide was kept under nitrogen and in the dark. A quench curve for each was constructed using the sample channels ratio method (16). \(^{14}\)C and \(^{35}\)S were estimated independently in a mixture of xylene/Triton X-100:2.5-diphenylhexane (700:300:5, v/v/v) (16), using the \(^{14}\)C window of a Philips PW 4040 liquid scintillation analyzer.

Materials—Coenzyme A was purchased from Boehringer Mannheim. Butyryl-CoA was prepared from CoASH and butyryl anhydride (17). Acetocetyl-CoA was prepared from CoASH and freshly vacuum distilled diketene (18, 19) and assayed in MgSO\(_4\) (20). Colloidal sulfur was produced from acidified sodium thiosulfate (21), stored in water, and vigorously shaken before use. Other chemicals were of the highest grade commercially available.

Persulfide Estimation—Samples were incubated for at least 30 min after addition of KCN to a final concentration of 5 mM. The thiocyanate produced was estimated by 460 nm absorbance after addition of "Sorbo's reagent" (22, 23) using a standard of ammonium thiocyanate. For this assay, \(\epsilon = 460 \text{ nm} = 4.0 \text{ mm}^{-1} \text{ cm}^{-1}\).

Taurine Analyses—Amino acid analyses with ninhydrin detection were performed on a single column Locarte bench model amino acid analyzer, interfaced to an MP6000-based integration computer. Samples were first dialyzed against 1 mM KPi, pH 7, and then hydrolyzed in 6 M HCl and 5% dimethyl sulfoxide (24) at 110 °C for 24 h. Under these conditions, CoA breaks down to give stoichiometric amounts of taurine and \(\beta\)-alanine (9). The exact taurine content for each sample was estimated in two consecutive analyzer runs, one (10 nmoles) to allow accurate estimation of taurine and the second (1 n mole) for estimation of the other amino acids. The taurine/proline ratio served as a convenient estimation of the protein CoA content expressed in all cases per mole of enzyme subunit.

Reconstitution of Apoprotein with Flavin Analogs—The apoprotein of general acyl-CoA dehydrogenase was prepared by the method of Mayer and Thorpe (25). Reconstitutions were performed at 4 °C or 25 °C for 1-2 h in 50 mM KPi, pH 7.6, using 25-36 mM apoprotein subunits and 1-2 eq of flavin analog. Unbound flavin was removed by gel filtration or dialysis against 50 mm KPi, pH 7. Details of the conditions will be published elsewhere.

RESULTS AND DISCUSSION

Fig. 1 shows the spectrum of yellow butyryl-CoA dehydrogenase and the complex produced after aerobic incubation of the yellow form with CoASH and Na\(_2\)S. The latter spectrum is indistinguishable from the published spectrum of native green butyryl-CoA dehydrogenase (3). Various forms of sulfur when added to yellow butyryl-CoA dehydrogenase together with CoASH are found to "green" the enzyme at markedly different rates with no change in activity in the dye-coupled catalytic assay. Qualitatively, the slowest greening is seen when Na\(_2\)S is added to a mixture of CoASH and yellow enzyme; after 15 min, the \(A_{335}/A_{430}\) ratio reaches 0.43. However, at equivalent concentrations, if Na\(_2\)S and CoASH are first incubated together for 15 min, before adding to the enzyme, the \(A_{335}/A_{430}\) ratio reaches 0.43 after 2 min. This strongly implies a reaction between the CoASH and S\(^2-\) before binding to the enzyme. Flowers of sulfur (a \(S_n\) species which exists as \(S_n\) rings) dissolve in a solution of Na\(_2\)S to give a mixture of linear polysulfanes (21)

\[
\text{HS}^- \rightarrow \text{HS}_n^- \quad \text{rapid}
\]

where \(n = 2, 3, 4 \text{ etc}, \text{ dependent on the concentration of S}^2-\).

If an aliquot of this mixture is added to yellow butyryl-CoA dehydrogenase and CoASH, relatively rapid greening occurs; the \(A_{335}/A_{430}\) ratio reaches 0.48 after 5 min. Furthermore, a suspension of colloidal sulfur in water, when added to yellow butyryl-CoA dehydrogenase and CoASH gives an \(A_{335}/A_{430}\) of 0.37 after 5 min. An \(S_n\) species of some sort is therefore clearly implicated in the greening reaction.

In control experiments, 1 mM Na\(_2\)S was incubated with two different samples of yellow butyryl-CoA dehydrogenase without any added CoASH. Unexpectedly, the spectrum of one sample after incubation showed an \(A_{335}/A_{430}\) ratio of 0.28 (52% greening). Amino acid analysis showed, however, that some endogenous CoA remained bound even after extensive anaerobic dialysis and that this sample of enzyme still contained 0.60 mol of CoA/mol of enzyme subunit. The second sample gave an \(A_{335}/A_{430}\) ratio of 0.13 (24%) with 0.30 mol of CoA bound/mol of enzyme subunit. These data indicate that CoASH is a prerequisite for greening and that approximately 1.2 mol of CoA/mol of enzyme subunit would be required to produce fully green enzyme (see later).

Similar experiments with general acyl-CoA dehydrogenase (which contains no bound CoA after purification (12)) showed slow greening when Na\(_2\)S was incubated with a mixture of CoASH and the enzyme. As with butyryl-CoA dehydrogenase, rapid greening was observed after prior incubation of CoASH and Na\(_2\)S, or when the polysulfane solution replaced the Na\(_2\)S. The maximum \(A_{335}/A_{430}\) ratio achieved for general acyl-CoA dehydrogenase was only 0.22. Furthermore, this complex is less stable than that of butyryl-CoA dehydrogenase; the \(A_{335}/A_{430}\) ratio falls to one-half after 20 h in aerobic 100 mM KPi, buffer, pH 7.6, at 25 °C, Native general acyl-CoA dehydrogenase, with no bound or added CoASH, showed no greening with Na\(_2\)S alone.

Persulfide Formation in Free Solution—The postulated reaction between CoASH and Na\(_2\)S in free solution was investigated further. In the absence of enzyme, 10 mM Na\(_2\)S and 0.48 mM CoASH were incubated in 0.2 M NaOH. This was undertaken to observe spectral changes in the 330-340 nm region, indicative of persulfide formation (26). The increase of 0.044 at 335 nm after 3 h is equivalent to 0.14 mM persulfide (\(\epsilon = 335 \text{ nm} = 300 \text{ mm}^{-1} \text{ cm}^{-1}\), see Refs. 26 and 27). Lowering the pH to <3 completely abolished the 335 nm absorption band, again consistent with a persulfide (26). These changes are also seen to a lesser extent in 100 mM KPi, pH 7, i.e. under the conditions of the greening experiments described above.

Correlation between \[^{14}\text{C}]\text{CoASH Binding and Greening}—Fig. 2 shows a close correlation between increasing greenness and the amount of \[^{14}\text{C}]\text{CoA} incorporated. However, the sample of yellow butyryl-CoA dehydrogenase used, when incubated with Na\(_2\)S alone, gave an \(A_{335}/A_{430}\) ratio of 0.17. This is due, as mentioned above, to endogenous bound CoA which is difficult to remove entirely. This complicates the interpretation of these results, as it is unclear to what extent the added radioactive CoA exchanges with the bound
unlabeled CoA. Additionally, the nonlinear portion of the curve indicates some nonspecific (i.e. nongreening) binding of the [14C]CoA.

Stoichiometry of 35S Binding—Sixty-four nmol of yellow butyryl-CoA dehydrogenase, 1740 nmol of Na2S, and varying amounts of CoA up to 192 nmol were incubated until greening reached completion. Unbound 35S was removed by the treatment described in Fig. 2 for the removal of unbound [14C]CoASH. Unlike the incorporation of [14C]CoASH, however, the amount of 35S initially bound showed a poor correlation with greenness. The stoichiometry of 35S incorporation into the greened samples, when extrapolated to fully green enzyme, ranged from 1.2 to 2.7 mol of 35S/mol of butyryl-CoA dehydrogenase. For example, one butyryl-CoA dehydrogenase sample was 68% green and contained 1.72 mol of 35S/mol of enzyme subunit (2.5 mol of 35S/mol of green butyryl-CoA dehydrogenase). Another sample, treated in the original incubation with a different amount of CoASH, was 92% green and contained 1.19 mol of 35S/mol of enzyme subunit (1.3 mol of 35S/mol of green butyryl-CoA dehydrogenase). These seemingly inconsistent results suggested that some nongreening 35S was bound to butyryl-CoA dehydrogenase, possibly as sulfide or as persulfide. In keeping with this view, it was found that, when [35S]greened enzyme was simply stored for 10 days at 4°C in buffer at pH 7, a pronounced decline in the bound 35S occurred with little or no concomitant decrease in greenness (Table I). The nongreening, relatively weakly bound sulfide is presumably in equilibrium with free sulfide in solution which is then slowly lost to the air as gaseous H2S. Additionally, persulfides are normally unstable and readily form sulfide.

After storage, the stoichiometry of residual 35S bound to the enzyme was close to 1 mol/mol of fully green butyryl-CoA dehydrogenase (Table I).

Cyanide reacts with persulfides to give free thiocyanate (22, 23), so that if some nongreening 35S is present as persulfide, then incubation in KCN might be expected to accelerate the removal of bound 35S (to give free CNS-). Incubation of native butyryl-CoA dehydrogenase with 5 mM KCN has been shown to produce no degreening (3). Therefore, the [35S] greened butyryl-CoA dehydrogenase was incubated for two consecutive periods of 48 h in 5 mM KCN at 4°C (Table I). A more rapid decline in bound 35S was seen with no significant decrease in greenness. Analysis of fractions collected from a calibrated Sephadex G-25 column (Fig. 3) indicates release of both 35S2- and CN35S-. The nongreening 35S is therefore in the form of both sulfide and cyanolyzable sulfur. The resulting stoichiometry after two KCN treatments (Table I) is 1.26 mol of 35S/mol of butyryl-CoA dehydrogenase, extrapolated to fully green enzyme.

Presence of Cyanolyzable Sulfur in the Greening Ligand—Although green butyryl-CoA dehydrogenase is not degreened by 5 mM KCN, green general acyl-CoA dehydrogenase loses 15% of greenness after 18 h at 4°C in 5 mM KCN. Increasing the temperature to 25°C abolishes 50% of greenness in 5-6 h. Because the greening ligands in butyryl-CoA dehydrogenase and general acyl-CoA dehydrogenase are thought to be identical, experiments were performed to determine whether cyanolyzable sulfur became accessible on denaturation of butyryl-CoA dehydrogenase using both [35S]greened and native enzyme. For this purpose, a calibrated Sephadex G-25 column was set up (see Fig. 3). Samples of [35S]butyryl-CoA dehydrogenase containing 1.00 mol of 35S/mol of enzyme subunit, and with A310/A430 = 0.40, were denatured under various conditions by incubation for 12 h in (i) 3% trichloroacetic acid, (ii) 6 M guanidinium chloride at pH 7, (iii) NaOH added to pH 10, or (iv) 5 mM KCN and NaOH to pH 10. Samples were then separately applied to the calibrated column. Examination of collected fractions showed a 260 nm peak of denatured protein at 14 ml and a 450 nm peak of released FAD at 35 ml. 35S was distributed between the protein peak and the CoASH/S2- peak. In the case of (iv), 35S was also seen under the thiochinate peak. In considering the results from Table II, it is important to bear in mind that although this enzyme sample is 74% green, it contains 1.00 mol of 35S/mol of butyryl-CoA.

### Table I

| Sample | A310/A430 | % greenness | Moles 35S bound/mol butyryl-CoA dehydrogenase | Moles 35S bound/mol butyryl-CoA dehydrogenase extrapolated to A310/A430 = 0.54 |
|--------|-----------|-------------|-----------------------------------------------|------------------------------------------------------------------|
| Sample 1 | 0.49 | 91 | 1.22 | 1.45 |
|         | 0.47 | 87 | 0.97 | 1.12 |
| Sample 2 | 0.47 | 87 | 1.85 | 2.13 | Initial incorporation |
|         | 0.47 | 87 | 1.02 | 1.17 | After storage |
| Sample 3 | 0.53 | 98 | 1.87 | 1.91 | Initial incorporation |
|         | 0.53 | 98 | 1.40 | 1.43 | After first KCN treatment |
|         | 0.52 | 96 | 1.21 | 1.26 | After second KCN treatment |
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sulfur before denaturation is 0.84 mol of $^{35}$S/mol of butyryl-CoA dehydrogenase. This is in close agreement with the assay for thiocyanate in the collected fractions using "Sorbo's reagent," which gives 0.80 mol of $^{35}$S/mol of butyryl-CoA dehydrogenase loaded.

(d) The total recovery of $^{35}$S improved as the pH increased. This is consistent with the destruction of persulfides to give free sulfide and subsequent partial loss to the atmosphere as the pH is lowered.

Various samples of native butyryl-CoA dehydrogenase with different $A_{260}/A_{450}$ ratios were denatured in either 5 mM KCN with NaOH added to pH 10, or in 150 mM KCN, and then applied to a calibrated Sephadex G-25 column (Fig. 3). The collected fractions were then assayed for thiocyanate as before. The samples showed a content of cyanolyzable sulfur ranging from 1.3 to 2.5 mol of $^{35}$S/mol of enzyme subunit extrapolated to fully green enzyme. Native butyryl-CoA dehydrogenase, therefore, contains cyanolyzable sulfur, some of which is not associated with greenness. Samples of the yellow form of the enzyme denatured in 150 mM KCN showed less than 0.15 mol of cyanolyzable sulfur per mol of enzyme subunit.

These denaturation experiments strengthen the supposition that the greening ligand is a compound containing a CoA moiety and one cyanolyzable sulfur atom. It appears that the enzyme also contains variable quantities of sulfur species which are not associated with greening. The cyanolyzable component of this additional sulfur could be attached to cysteine residues in persulfide linkage. Conceivably there also may be significant formation of coenzyme A polysulfides.

**Binding of $^{35}$S to Bovine Serum Albumin and Pig Heart Lactate Dehydrogenase**—To examine the possibility that butyryl-CoA dehydrogenase might not be unique in binding sulfides "nonspecifically," separate solutions of bovine serum albumin and lactate dehydrogenase (7 mg ml$^{-1}$) were incubated for 24 h with 3 mM Na$_2^{35}$S. Unbound $^{35}$S was removed by gel filtration. Analysis of the treated proteins showed 0.4 mol of bound $^{35}$S/mol of lactate dehydrogenase and 1.3 mol of bound $^{35}$S/mol of bovine serum albumin. Similar results have been reported by Cavallini et al. (27) for a number of proteins and the formation of protein-bound persulfide was found to be correlated with the presence of disulfide bonds in the protein.
untreated proteins.

Treatment of Butyryl-CoA Dehydrogenase with Dithionite—Reducing native butyryl-CoA dehydrogenase with dithionite abolishes the 710 nm absorption band. A sample of [35S]greened enzyme was treated with dithionite, followed by dialysis against 10 mM dithionite at pH 7. Subsequent oxidation of the enzyme yielded the yellow form of the enzyme with only 5% of the original 35S remaining bound. If the sample was reoxidized without dialysis, and then dialysed aerobically, 13% of the original 35S remained unbound. While dithionite reduction followed by anaerobic dialysis removes some bound CoA from butyryl-CoA dehydrogenase, dithionite reduction followed by aerobic dialysis does not (3). These data, therefore, show that degreening by dithionite is not due to loss of bound CoA, but to removal of the cyanolyzable sulfur atoms of the greening ligand, possibly by sulfidolysis.

Replacement of the Greening Ligand—The addition of acetoacetyl-CoA to yellow butyryl-CoA dehydrogenase instantaneously produces a complex with a broad absorption band centered at 580 nm (7). An excess of acetoacetyl-CoA, when added to the green form of the enzyme, also gives a 580 nm band after a few seconds.2 Replacement of tightly bound ligand by an excess of another ligand in the acyl-CoA dehydrogenase has been well documented (7, 28). In the displacement experiments reported here, the procedure was as follows. A 10-fold excess of acetoacetyl-CoA was added to [35S]greened enzyme and the 710 nm and 580 nm were monitored. When each was constant, the sample was applied to a Sephadex G-25 column and fractions containing the enzyme were pooled. These fractions were counted for radioactivity and spectra were recorded. A reduction in bound [35S]CoASH proportional to the decrease in A710:A430 and concomitant rise in A580:A430 clearly showed replacement of the greening ligand. However, 35S was only partially displaced after binding of acetoacetyl-CoA. A reduction in the A710:A430 ratio of 0.31 (corresponding to displacement of 0.58 mol of the greening ligand per mol of enzyme subunit) was accompanied by a decrease in the bound 35S of only 0.30 mol/mmol of enzyme subunit. While the CoA moiety is clearly fully replaced, part of the cyanolyzable sulfur appears to remain. A possible explanation is that once the CoA persulfide is released from the enzyme, the cyanolyzable sulfur atom can migrate from the CoA sulfur to form similar persulfide links with cysteine residues by a simple exchange.

Stability of the Greening Ligand—Persulfides in free solution are unstable and at low pH readily break down to give sulfides (21). However, it is apparent that the green butyryl-CoA dehydrogenase complex is very stable—the greening ligand remains bound even after cold storage for several years, extensive dialysis, ammonium sulfate precipitation, and treatment in 3.5 M urea (3, 7, and Footnote 1). On the binding site, therefore, the persulfide linkage must some-
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(see "Experimental Procedures"). The results are shown in Table III, which lists the energy of the long wavelength band (expressed as $\Delta E_{\text{CT}}$, reciprocal of the wavelength maximum) and known oxidation-reduction potentials of the flavin analog. The charge-oxidation transition may involve either one- or two-electron transfer in the excited state, (40) so both the one-electron potential, for the couple $\text{FL}_{\text{ox}}/\text{FH}$ (where known) and the two-electron potential, $\Delta E_{\text{ox}}/\text{FH}$, are listed. It should be noted that while the correlation is reasonably good for $\Delta E_{\text{CT}}$ versus the two-electron potential for most of the flavins, the correlation is much better if the one-electron potential for 5-deaza-FAD is used. This is in keeping with the known thermodynamic instability of the 5-deazaflavin radical (39).

The correlation observed is that expected for a charge-transfer interaction in which oxidized flavin serves as the charge-transfer acceptor (40). The donor is most probably the ionized persulfide species, CoA--S$^-$--S$,^-$, and precedent for a --S$^-$ -- FAD charge-transfer complex comes from studies with lipoamide dehydrogenase and glutathione reductase. The absorptions generated on two-electron reduction of these flavoenzymes have been attributed to a charge-transfer interaction between a cysteinyl thiolate residue and oxidized FAD (41-45). This absorbance is abolished on protonation of the thiolate (44, 45).

**Physiological and Metabolic Significance**—Butyryl-CoA dehydrogenases from most mammalian sources (4, 28, 46) and from *M. elsdenii* (3) are green. Glutaryl-CoA dehydrogenase, a similar flavoprotein from *Pseudomonas fluorescens* is also green (29). It is most likely that the greening ligand in each is a tightly bound CoA persulfide. The in vivo formation of such a ligand must involve some form of sulfur donor.

In the case of butyryl-CoA dehydrogenase in *M. elsdenii*, which is a strict anaerobe, the CoA persulfide could be produced by the enzyme itself according to the following scheme:

\[
\begin{align*}
S^2^- & \quad \text{FAD}_{\text{ox}} \quad \text{butyryl-CoA} \\
\downarrow \\
S^2^- + \text{CoASH} + \text{butyryl-CoA} & \quad \rightarrow \text{green butyryl-CoA dehydrogenase} \\
\end{align*}
\]

In the course of the experiments reported here, it was observed that a large excess of sulfide can reduce both general acyl-CoA dehydrogenase and butyryl-CoA dehydrogenase as required for the above scheme. Furthermore, *M. elsdenii* releases copious quantities of hydrogen sulfide in culture (15).

In the case of mammalian tissues, a more specific sulfur donor may be involved, since even low levels of sulfide are toxic (21). A well-documented sulfur donor is rhodanese (e.g., Refs. 21, 47, and 48) which is found in large quantities in liver and kidney (21). No physiological function has yet been found for this enzyme, but it is known to catalyze a variety of reactions in vitro (21), such as

\[
\begin{align*}
\text{Rhodanese} + 2 \text{S}^- + \text{SO}_4^{2-} & \rightarrow \text{RS(OH)}_2^- \quad \text{Rhodanese} - \text{S} + 2 \text{CN}^- \\
\end{align*}
\]

Rhodanese is mainly associated with the mitochondria in vivo (21).

3-Mercaptopyruvate sulfurtransferase is present in rat liver and other tissues (21). The enzyme converts 3-mercaptopyruvate to pyruvate and elemental sulfur:

\[
\begin{align*}
\text{CH}_2\text{SH} & \quad \rightarrow \quad \text{CH}_3\text{COOH} \\
\end{align*}
\]

this reaction may involve the formation of an enzyme persulfide (49).

The CoA persulfide is tightly bound to butyryl-CoA dehydrogenase and, in vivo, it may therefore present a block to dehydrogenase activity unless a sufficient quantity of substrate is present to displace the greening ligand. It is therefore possible that the CoA persulfide could serve as a regulatory ligand for butyryl-CoA dehydrogenase; the enzyme would be "activated" (by displacement of the CoA persulfide, an "inhibitor") only when a sufficient, threshold quantity of substrate was present.

The discovery that a CoA persulfide is responsible for the 710 nm absorption band in native butyryl-CoA dehydrogenase provides a satisfying solution to a number of long standing puzzles. The possible physiological significance of such a tightly bound ligand is intriguing and needs to be fully explored. Equally intriguing are the molecular mechanisms by which the enzyme is "greened" in vivo and by which the normal unstable persulfide is stabilized when complexed with butyryl-CoA dehydrogenase.

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**Table III**

Dependence of charge-transfer transition on flavin oxidation-reduction potential

Apoprotein was reconstituted with the analogs shown and spectra were recorded in 0.7 ml of 50 mM KP, pH 7.6 at 10°C, before and after the addition of 10 µl of a mixture of 20 mM CoASH and 20 mM Na$_2$S, which had been preincubated for 1 h at room temperature.

| Flavin       | $\lambda_{\text{max}}$ | $\Delta E_{\text{CT}}$ | $E\text{pH}7\text{ Fl}_{\text{ox}}/\text{FH}$ | $E\text{pH}7\text{ Fl}_{\text{ox}}/\text{FH}$ |
|--------------|------------------------|-------------------------|---------------------------------------------|---------------------------------------------|
| 5-Deaza-FAD  | ~650                   | ~20,000                 | ~275*                                       | ~650*                                       |
| 6-Hydroxy-FAD| ~15,500                | ~260*                   |                                             |                                             |
| 1-Deaza-FAD  | ~15,400                | ~280*                   |                                             |                                             |
| 5-Deaza-FAD  | ~13,700                | ~152*                   |                                             |                                             |
| 2-Thio-FAD   | ~12,000                | ~126*                   |                                             |                                             |
| 6-Hydroxy-FAD| ~15,600                | ~260*                   |                                             |                                             |
| 8-Chloro-FAD | ~14,100                | ~208*                   |                                             |                                             |
| FAD          | ~710                   | ~239*                   |                                             |                                             |

* Ref. 34.  
* Ref. 35.  
* Ref. 36.  
* P. Hemmerich, unpublished data.  
* Ref. 37.  
* Ref. 38.  
* Ref. 39.
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