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Evidence from Fourier Transform Infrared Spectroscopy for Polarization of the Carbonyl of Oxaloacetate in the Active Site of Citrate Synthase†

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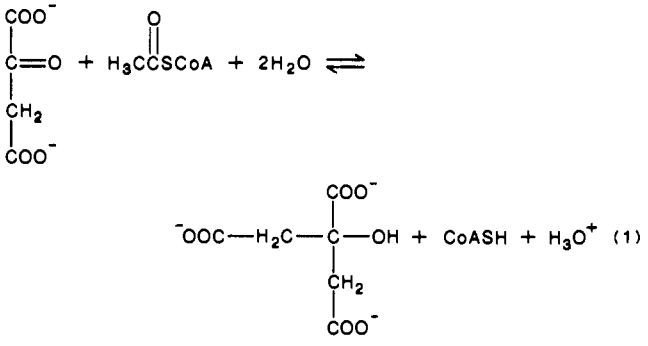
ABSTRACT: The infrared spectrum of oxaloacetate bound in the active site of citrate synthase has been measured in the binary complex and in the ternary complex with the acetyl coenzyme A (CoA) enolate analogue carboxymethyl-CoA. The carbonyl stretching frequency of oxaloacetate in binary and ternary complexes is found at 1697 cm^{-1}, a shift of 21 cm^{-1} to lower frequency relative to that of the free ligand. The line widths of the carbonyl absorption in enzyme complexes differ from that of the free ligand, decreasing from a value of 20 cm^{-1} for the free ligand to 10 cm^{-1} in the binary complex and 7 cm^{-1} in the ternary complex with carboxymethyl-CoA. The integrated absorbance of the carbonyl absorption in these enzyme complexes is significantly increased over that of the free ligand at the same concentration, increasing ~2-fold in the binary complex and ~3-fold in the ternary complex. These results indicate strong polarization of the carbonyl bond in the enzyme–substrate complexes and suggest that ground-state destabilization is a major catalytic strategy of citrate synthase.

Citrate synthase (EC 4.1.3.7) catalyzes the condensation of oxaloacetate (OAA) with acetyl coenzyme A (CoA) to form citrate (eq 1). The chemical mechanism is thought to involve



generation of the carbanion (enolate) of acetyl-CoA, which condenses with the carbonyl of OAA to form *S*-citryl-CoA as an intermediate (Eggerer, 1965; Weidman & Drysdale, 1979; Eggerer & Remberger, 1963; Bayer et al., 1981). It has been proposed that the carbonyl of OAA could interact with an electrophilic residue resulting in polarization of the C=O bond with substantial positive charge development at the carbonyl carbon (Srere, 1966). Polarization of the carbonyl would enhance the reactivity of the carbonyl carbon toward carbanion addition.

We have previously reported (Kurz et al., 1985) very large C-13 chemical shift changes (+6.8 ppm, downfield) for the carbonyl of OAA upon binding to the enzyme, which we have interpreted as evidence for polarization of the carbonyl of OAA in the active site. However, our interpretation of even these large changes in ^{13}C shifts as resulting from carbonyl bond polarization has been questioned (Malthouse, 1986). We now present independent evidence from Fourier transform infrared spectroscopy (FTIR) supporting the validity of our interpretation of the NMR results.

Materials and Methods

Crystalline citrate synthase was a product of Sigma Chemical Co., St. Louis, MO. Enzyme crystals were first dissolved in 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.50. After extensive dialysis to remove $(NH_4)_2SO_4$, enzyme samples were concentrated to ~300 mg/mL (0.5 mL) by centrifugation in a CF25 Centriflo concentrator (Amicon Corp.). The sample was rediluted with 4.5 mL of 50 mM Tris-DCl, pD 7.90, in D_2O and reconcentrated to 0.5 mL, repeating this procedure 3 times to ensure complete exchange of solvent protons. The sample was removed from the membrane cone, which was rinsed with 0.5 mL of buffer, to obtain a final concentration of 1–3 mM active sites.

For experiments with the OAA–enzyme complex, sample preparation included a Sephadex G-25 purification step to remove small molecule contaminants (pyruvate, α-ketoglutarate, etc.). After concentration of the enzyme as described above, a 10% molar excess of OAA in D_2O was added to form the binary complex. The sample was applied to the top of a 2.8-mL column of Sephadex G-25 equilibrated in 50 mM Tris-DCl, pD 7.9 (in D_2O), and centrifuged briefly to

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recover the protein. The concentration of the OAA–citrate synthase complex was determined by the DTNB method (Srere, 1969).

[2-^{13}C]OAA was prepared as described previously (Kurz et al., 1985) except that D_2O was used for all solutions. Carboxymethyl-CoA was prepared according to Bayer et al. (1981). After the Sephadex G-10 desalting, the sample was carefully neutralized and lyophilized. After a second lyophilzation from D_2O, the sample was dissolved in D_2O buffer.

Infrared measurements were made at 1-cm^{-1} resolution on a Nicolet 7199 Fourier-transform infrared spectrometer. The sample cell was a CaF_2 flow-through cavity cell with a 0.1-mm path length (Spectra-Tech Corp.). Sample was introduced by displacement of the previous solution via miniature Hamilton valves mounted on the cell compartment cover. The solution entered from the bottom of the cell (0.3 mm i.d. Teflon tubing) and exited from the top (0.5 mm i.d. Teflon tubing). Replacement of one sample with the next required 0.2–0.4 mL. Development of bubbles during sample introduction was avoided by gentle syringe suction via the exit tube while keeping the entrance tube submerged in the sample solution. After the cell was placed in the sample compartment, the entire spectrometer was purged with N_2 for at least 12 h before use. For 1 h prior to and during use, the purge rate was increased. The increased purge rate and the ability to change samples without opening the cell compartment were required to reduce the contribution of water vapor to the spectrum to an acceptable level. There are several large water vapor peaks in the region of interest ($\sim$1700 cm^{-1}), and these were removed by subtraction of a water vapor absorbance spectrum. In the spectrum shown in panel C of Figure 1, the intensities of the vapor peaks were $\sim$10% of the OAA peak. For single-beam spectra, 800 interferograms were averaged, requiring an acquisition time of $\sim$22 min. After Fourier transformation, the transmission spectrum of each sample was ratioed to that of buffer and the result converted to absorbance. Difference spectra were constructed by subtraction of the absorption spectrum of an appropriate blank. The composition of the blank was identical with that of the sample except for an additional $\sim$15% molar excess of acetyl-CoA, which was required for conversion of the OAA to citrate. Citrate, acetyl-CoA, and CoA do not have any absorbances in the frequency region of interest. When required by cumulative pipeting errors (causing small concentration differences between sample and blank), small adjustments (2–3%) were made in the amount of blank subtracted in order to obtain a flat base line above 1740 cm^{-1}. Relative absolute intensities were calculated from the areas of the absorption peaks. Since no analytical function is available for IR band shapes, the return to the base line was approximated.

Results

The infrared spectrum of free oxaloacetate ($\sim$3 mM) containing pyruvate ($\sim$0.4 mM) is shown in panel A of Figure 1. The ordinate is expanded 4.3 times relative to panels C and D. Oxaloacetate is unstable in aqueous solution, decarboxylating to pyruvate at a rate of $\sim$0.2 mM/h under these conditions. The concentrations reported in this experiment are the average of the results of enzymatic assays performed before and after the spectrum of the solution was measured. Subtraction of a spectrum of pyruvate, maximum at 1707 $\pm$ 0.5 cm^{-1}, reveals that OAA has its maximum absorption at 1718 $\pm$ 1 cm^{-1}. The line width (width at half-height) is 20 $\pm$ 1 cm^{-1}. Fortunately, the stability of OAA is greatly increased when it is bound to citrate synthase, and no decomposition occurs during the time required for the experiments

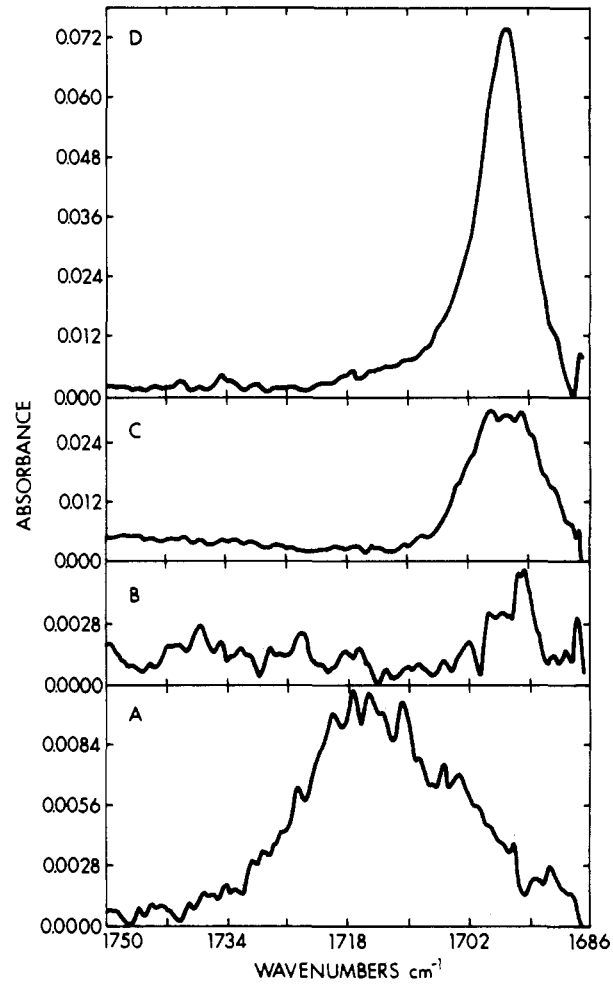


FIGURE 1: Infrared spectra of oxaloacetate, free and in complexes with citrate synthase. (Panel A) Infrared spectrum of free oxaloacetate ($\sim$3 mM) containing pyruvate ($\sim$0.4 mM) in 50 mM Tris-DCl, pD 7.9. Ordinate expanded 4.3 times relative to those of panels C and D. (Panel B) Infrared spectrum of the binary complex of [2-^{13}C]-oxaloacetate with citrate synthase, 1.7 mM in 50 mM Tris-DCl, pD 7.9. Ordinate expanded 4.3 times relative to those of panels C and D. (Panel C) Infrared spectrum of the binary complex of oxaloacetate with citrate synthase, 2.50 mM in 50 mM Tris-DCl, pD 7.9. (Panel D) Infrared spectrum of the ternary complex of oxaloacetate and carboxymethyl-CoA with citrate synthase, 2.50 mM in Tris-DCl, pD 7.9.

with enzyme complexes (Kurz et al., 1985).

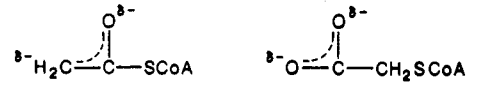
Panel C of Figure 1 shows the spectrum of the binary complex of oxaloacetate with citrate synthase. No unbound OAA is present in these experiments (the K_{diss} in 4 M urea is estimated to be 1–5 μM; Srere, 1966; Johansson et al., 1973). The maximum absorption occurs at 1697 $\pm$ 0.5 cm^{-1} with a line width of 10 $\pm$ 1 cm^{-1}. No absorbance in the region of the free ligand absorption is observed. The change in frequency compared to that of the free ligand is -21 cm^{-1}.

The new absorption in the enzyme complexes lies in a region of relatively high (0.24–0.4 OD at 1697 cm^{-1} depending on concentration) and rapidly increasing absolute absorbance (1.7–2.5 OD at 1686 cm^{-1}, the extreme edge of Figure 1). Several controls are therefore necessary (data not shown). (1) Since independently prepared samples subtract almost exactly to leave a relatively noise-free base line, we have not exceeded the dynamic range of the instrument. (2) The technique has adequate sensitivity in the region of interest. We have examined the carbonyl spectrum of pyruvate at a concentration of 1 mM (2.5 times lower than the concentrations of the experiments reported in panels C and D of Figure 1) in the

presence of equimolar citrate synthase, and we observe the carbonyl absorption of pyruvate at 1707 cm^{-1}. Pyruvate does not bind to the enzyme at these concentrations. Free pyruvate has roughly half the molar extinction coefficient of the OAA–citrate synthase absorption. Thus, we can detect weak absorptions even ~5 times lower in intensity than that observed in the OAA–enzyme binary complex. However, the fact remains that the new absorption observed in the OAA–enzyme complexes is at the extreme edge of reliable measurement. As a consequence, the line shape of the absorption may be somewhat distorted on the low-frequency side.

The new absorption in these difference spectra could arise from the enzyme or the ligand; therefore, the enzyme complex with [2-^{13}C]OAA was prepared and its spectrum measured (panel B). New absorptions in this region could have arisen from shifts in enzyme carboxylate/amides absorptions as a consequence of conformation changes upon complexation as has been reported for aldolase (Belasco & Knowles, 1983). However, no significant absorption is found at 1697 cm^{-1} where the presumptive [2-^{12}C]carbonyl stretch was observed. On the basis of the effect of ^{13}C subsitution in the carbonyl of pyruvate on the carbonyl frequency, ^{13}C substitution at C-2 of OAA is expected to shift the carbonyl absorption ~40 cm^{-1} to lower frequency where it will be obscured by the intense protein absorption and where meaningful subtraction cannot be performed. The small positive feature remaining at ~1700 cm^{-1} arises from the presence of ~10% ^{12}C isotopic impurity. (The ^{12}C absorption in [^{13}C]OAA–citrate synthase complexes is more evident in the carboxymethyl-CoA ternary complex as a result of the increased carbonyl intensity found in that complex.) These data indicate that the absorption observed in the ^{12}C spectrum arises from the carbonyl of OAA and not from absorption shifts in the protein amide/carboxylate spectrum that might have accompanied binding of substrate.

Carboxymethyl-CoA is an analogue of the carbanion/enolate of acetyl-CoA, a proposed intermediate in the citrate



acetyl-CoA enolate carboxymethyl-CoA

synthase reaction. As would be expected for an intermediate/transition-state analogue inhibitor, it binds very tightly to the enzyme–OAA complex (K_{diss} = 0.07 μM; Bayer et al., 1981).

The infrared spectrum of the ternary complex of oxaloacetate and carboxymethyl-CoA with citrate synthase is shown in panel D. The absorption occurs at the same frequency as in the binary complex. The half-width is significantly smaller, 7 ± 1 cm^{-1}, and the absolute absorbance is considerably greater. No absorption attributable to free carboxymethyl-CoA (1625 cm^{-1}) occurs in this region. Experiments with [2-^{13}C]OAA reveal, as in the binary complex, that all of the absorption in this region is attributable to the OAA carbonyl and not to complexation-induced shifts in protein, other OAA, or carboxymethyl-CoA absorptions (data not shown).

Frequency and intensity data for OAA and its complexes with citrate synthase are summarized in Table I. In the calculation of the intensities of protein-bound OAA relative to the free ligand, no correction was made for the fact that in neutral aqueous solution OAA is present as ~90% keto form with the remainder in hydrate and enol forms (Kokesh, 1976). The true intensity increase for the carboxymethyl-CoA complex may be larger than the observed factor of 3.0; this complex tends to plate out on the cell walls, and subsequent washing with buffer may not have been sufficient to ensure an exact blank spectrum upon introduction of the sample of complex plus acetyl-CoA.

Table I: Infrared Data for the Carbonyl Absorption of Oxaloacetate

	frequency (cm^{-1})[a]	line width (cm^{-1})[b]	intensity (integrated absorbance)
free ligand	1718	20	1.0
binary complex	1697	10	2.0
ternary complex	1697	7	≥3.0

[a] Errors (standard deviations of replicate measurements) are ±0.5 cm^{-1}. [b] Errors (standard deviation of replicate measurements) are ±1 cm^{-1}.

Discussion

Frequency Shift. Upon binding to citrate synthase, the carbonyl stretching frequency of oxaloacetate is found to decrease 21 cm^{-1}. The simplest explanation of this result is polarization of the carbonyl by active site electrophile(s). This interpretation is in accord with ^{13}C NMR studies (Kurz et al., 1985), crystallographic studies (Remington et al., 1982; Wiegand et al., 1984), and model studies on the effects of protonation and polarization (by Lewis acids) on carbnyl frequencies (Clemett, 1970; Bellamy, 1968). Similar decreases in carbonyl frequency have been observed when dihydroxyacetone phosphate binds to triosephosphate isomerase and have been similarly interpreted (Belasco & Knowles, 1980).

The frequency shift upon binding could conceivably be a consequence of a change in the relative orientations of the carboxylate and carbonyl dipoles. This possibility can be modeled by solution spectra of α-halogenated ketones, which have two carbonyl absorption bands separated by about 20 cm^{-1} (Bellamy & Williams, 1957). One of these is close in frequency to the single vapor phase absorption and is attributable to a conformation in which the carbonyl and carbon–halogen dipoles are opposed and well separated in space. The absorption at higher frequency is attributable to a conformation in which the dipoles are more closely aligned and near to each other. The increase in frequency in this latter conformation is readily understood to arise from a mutual induction of charges that reduces the carbonyl bond polarity. However, it is highly improbable that the frequency shifts we have observed arise from OAA conformer selection. In OAA, the orientation of the α-carboxylate dipole relative to that of the carbonyl is fixed by the sp^2 hybridization of carbons 1 and 2 and therefore could not be responsible for the observed frequency shift. In the bound OAA, dipole–charge interaction with the 4-carboxylate is unlikely to be significantly less (in order to lower the carbonyl frequency) than it is in free solution. In solution, electrostatic repulsion between the 1- and 4-carboxyaltes should favor maximum separation of the C-1 and C-4 carboxylates. The crystallographic studies of OAA bound to citrate synthase (Wiegand et al., 1984) show a slightly less than maximal separation between these carbons, which would tend to raise the carbonyl frequency rather than to lower it as is observed.

Thus, it is not easy to escape the conclusion that these results indicate an increased polarization of the carbonyl of oxaloacetate upon binding to the active site of the enzyme. We favor the argument either that the highly polar nature of the citrate synthase active site, containing several obligatorily and potentially positively charged residues (Wiegand & Remington, 1986), in itself causes an induction of charges in the carbonyl, which increases its polarity, or that there exists a specific interaction between the carbonyl and an enzymatic electrophile or proton. A semiquantitative estimate of the

magnitude of the effect [based upon the protonation of acetone as a model and entirely analogous to the arguments used previously by us in discussion of the NMR studies (Kurz et al., 1985) and by Belasco and Knowles (1980) in discussion of their IR studies with triosephosphate isomerase] supports a significant contribution to the catalytic efficiency of citrate synthase by substrate destabilization. This frequency shift indicates a change in the C=O bond order from ~2 to ~1.7.

Changes in Band Shape. The band shape of IR absorption bands in condensed phases is attributable to the multiplicity of intermolecular interactions (with solvent) and/or intramolecular interactions (via different molecular conformations) which result in slight frequency shifts and the consequent smearing and broadening of the rotational bands that would be present in the vapor spectrum. It has previously been noted (Fisher et al., 1980) that IR absorption bands of enzyme-bound species tend to be narrower than those of the free ligands. This may be reasonably attributed to the lesser degree of conformational freedom experienced by specifically bound ligands so that there are fewer rotational states accessible and fewer, more specific intermolecular (protein–ligand) interactions. This was expected for the present case since NMR data (Kurz et al., 1985) indicate that the bound OAA is immobilized on the enzyme and is constrained to rotate with it. Furthermore, the tendency toward even narrower lines observed in ternary complexes is in accord with the tighter binding of OAA in these complexes and the likely consequent increased immobilization.

Changes in Intensity. We have little to guide us in explaining the large intensity changes. Although frequency shifts attributed to carbonyl polarization have been observed previously in the triosephosphate isomerase complex with dihydroxyacetone phosphate and in the yeast aldolase complex with glyceraldehyde 3-phosphate (Belasco & Knowles, 1983, 1985), no intensity comparisons with free ligand are available for those two examples. For triosephosphate isomerase, the internal equilibrium constant (relative proportions of bound substrate and product) is not known with certainty, and no comparison with the absorption intensity of free ligand is presently possible. For aldolase, glyceraldehyde 3-phosphate is extensively hydrated in aqueous solution, and the carbonyl absorption of the free ligand is very weak.

The intensity of IR absorptions is proportional to $\delta\mu/\delta Q$, the change in dipole moment with the change in normal coordinate. For a carbonyl group, μ can be regarded as the product of the partial charge on the carbonyl carbon (and oxygen) times the length of the carbon–oxygen bond. The normal coordinate Q is approximately the same as the valence coordinate r (where r is the C=O bond length). To a first approximation then, the greater the initial polarization of the carbonyl bond, the greater the change in dipole moment with r and the greater the IR intensity. Model studies of the effects of increased hydrogen bonding on the intensity and frequency of the carbonyl stretching absorption of acetone (Tsubomura, 1956) indicate that this picture is qualitatively correct. It is possible then that the increased intensity (integrated absorbance) observed for OAA complexes with the enzyme is a consequence of the polarizatiion of the C=O bond upon binding and that the further increase in intensity in the ternary complex results from greater polarization. However according to this model (Tsubomura, 1956), we would have expected also to see a further frequency shift accompanying the intensity increase.

Failure to observe additional frequency shifts in the ternary complex might be explained in two ways. First, we cannot say whether we have observed all the OAA absorptions. The observed frequency is at the edge of reliable observation and any absorption at lower frequency would be obscured by the protein (as in the ^{13}C experiments). Second, we cannot neglect the possibility that the normal coordinate describing the vibration of the bound ligand is significantly different from that of the free ligand. For instance, the bound carbonyl could be part of an extensive, vibrationally coupled hydrogen-bonding system. The effective separation of the formal charges in the system would then be greater than that in the free ligand and would thus increase the intensity of the absorption. In some conjugated carbonyl systems, the contribution of charge-separated resonance forms has been invoked to explain increases in IR intensities (Barrow, 1953). In these same systems, as in ours, there was a lack of correlation between changes in intensities and frequencies.

Table II: Comparison of IR and NMR Data

	$\Delta\delta$ (ppm)	$\Delta\nu$ (cm^{-1})
binary complex	+4.3[a] (14%)[b]	−21 (21%)[b]
ternary complex	+6.8[a] (23%)[b]	−21 (21%)[b]
protonated acetone	+30[c]	−100[d]

[a] Kurz et al., 1985. Downfield shifts from that of the free ligand. [b] Percent change relative to total change observed for acetone protonation. [c] Tiffon & Dubois, 1978. [d] Clemett, 1970.

Comparison of NMR and IR Results. Qualitatively, both the NMR and the IR data strongly support the proposition that substantial polarization of the OAA carbonyl occurs upon binding to the active site. However, there are several quantitative differences as discussed below (Table II).

The carbonyl chemical shift increases in a series of ternary complexes, reaching its maximum downfield shift in the ternary complex with carboxymethyl-CoA. This latter result has been interpreted to mean an increase in polarization of the C=O bond, reaching its maximum extent in the carboxymethyl-CoA ternary complex (Kurz et al., 1985). We expected to see then an analogous progressive decrease in the carbonyl frequency in ternary complexes. This was not observed as shown in Table II. Furthermore, the frequency shift of the absorption band for the binary complex is near the maximum expected for the degree of polarization indicated by the NMR data on the carboxymethyl-CoA ternary complex (using IR and NMR data on the protonation of acetone as a model, Table II). However, the increase in integrated absorbance between the ternary and binary complexes might indicate an increased polarization in the carboxymethyl-CoA complex since there is no theory to lead us to expect a linear relation between frequency and intensity changes. Alternatively, part of the frequency shift observed in NMR experiments may be influenced by magnetic factors not detectable in IR experiments.

In addition, the NMR data (Kurz et al., 1985) suggest that there is a chemical exchange process in the binary complex not involving the free ligand that is at least partially averaged on the NMR time scale. While this exchange process seems to involve primarily the 4-carboxylate, the carbonyl appears to be involved to some extent. We hoped to exploit the difference between NMR and IR time scales to detect multiple IR absorption peaks for the binary complexes. From the NMR data, we expected to see more than one IR absorption for the binary complex, one or more of which might indicate a lesser degree of polarization than that found in the carboxymethyl-CoA complex. However, we have observed only one IR absorption. The increase in intensity between binary and ternary complexes might indicate that absorption bands too weak to detect in the binary complex have coalesced. In

addition, since our window for observation is severely limited, carbonyl absorptions with greater frequency shifts than that which we have observed could be present in some of these complexes. The possibility also exists that the chemical exchange process observed in NMR experiments is not one between states differing in carbonyl polarization and thus does not substantially effect the IR frequency.

Summary. Significant polarization of the carbonyl of OAA occurs upon binding to the active site of citrate synthase. This is the first observation of substrate destablization by both NMR and IR.

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Regulation of the Butyryl-CoA Dehydrogenase by Substrate and Product Binding†

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ABSTRACT: Until now, workers in the field of fatty acid metabolism have suggested that the substrates are isopotential with the enzymes and that the reactions are forced to completion by the formation of charge-transfer complexes [Gustafson, W. G., Feinberg, B. A., & McFarland, J. T. (1986) *J. Biol. Chem. 261*, 7733–7741]. To date, no experimental evidence for this hypothesis exists. The work presented here shows that the butyryl-CoA/crotonyl-CoA couple is not isopotential with the enzymes with which it interacts. The potential of the butyryl-CoA/crotonyl-CoA couple ($E^{\circ\prime}$ = –0.013 V) is significantly more positive than the potential of either of the enzymes with which it interacts, bacterial butyryl-CoA dehydrogenase ($E^{\circ\prime}$ = –0.079 V) and mammalian general acyl-CoA dehydrogenase ($E^{\circ\prime}$ = –0.133 V). These data imply that the regulation of enzyme potential is essential for any electron transfer from substrate to enzyme to occur in mammalian or bacterial systems. In support of this assertion, a significant shift in potential for bacterial butyryl-CoA dehydrogenase (an analogue of the mammalian enzyme) in the presence of butyryl-CoA and crotonyl-CoA is reported. The potential is shifted positive by 60 mV. Larger potential shifts will undoubtedly be observed with the mammalian enzyme, which would be consistent with the catalytic direction of electron transfer.

We were interested in studying the interactions of the fatty acyl-CoA's[1] with their dehydrogenases in bacterial and mammalian systems. From extensive work done on GACD, we know that this enzyme binds a wide variety of substrates but is reduced to different degrees by different substrates (Thorpe et al., 1979). The better substrates reduce the enzyme, and their products also form charge-transfer bands with the reduced enzyme. Poorer substrates partially reduce GACD. Binding is thought to play an important role in the extent of reduction. Thorpe et al. (1979) have found that with optimal substrate the degree of enzyme reduction is controlled by the relative tightness of binding of the substrate and product to the oxidized and reduced enzyme, respectively. This implies that, for titrations with optimal substrate, the product binds tighter to the reduced enzyme, favoring reduction of FAD,

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[1] Abbreviations: CoA, coenzyme A; BCD, butyryl-CoA dehydrogenase; GACD, general acyl-CoA dehydrogenase; 8ClRF, 8-chlororiboflavin; PYC, pyocyanine; ETF, electron-transfer flavoprotein; CCoA, crotonyl-CoA; BCoA, butyryl-CoA; $E^{\circ\prime}$, midpoint potential; IDS, indigodisulfonate; ITRS, indigotrisulfonate; FACoA, *trans*-β-2-furylacryloyl-CoA; FPCoA, β-2-furylpropionyl-CoA; MV, methylviologen; $\hat{E}^{\circ\prime}$, conditional equilibrium potential; SHE, standard hydrogen electrode. Anything with subscript ox or red refers to the oxidized or reduced form of that species.

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