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Fluorescence Detection of Increased Local Flexibility Induced by Coenzyme A in Succinyl-CoA Synthetase from *Escherichia coli*†

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ABSTRACT: The enzyme succinyl coenzyme A synthetase catalyzes the formation of succinyl coenzyme A (CoA) from succinate, CoA, and a nucleoside triphosphate. The enzyme has been labeled with dansyl chloride in both the α and β subunits to give an almost fully active fluorescent conjugate. Addition of CoA at 1 mM caused the polarization of the conjugate to fall from ~0.275 to ~0.145. Control studies indicated that this result was not due to effects such as dissociation of the protein, release of bound label, or changes in the lifetime of the bound label. The other substrates, ATP·Mg^{2+}, succinate, or ADP·Mg^{2+}, gave a polarization decrease when added to the conjugate, but to a much smaller degree compared to the effect of CoA. For example, the maximum decrease with the other substrates was observed with ADP·Mg^{2+} where the polarization only fell to 0.245 at 5 mM ADP·Mg^{2+}. Comparison with model compounds indicates that the effect of CoA is specific. Analysis of the polarization data gives rotational relaxation times for the conjugate which are smaller than that expected for the protein considered as a sphere, in both the presence and absence of CoA. Perrin–Weber plots in the presence of CoA are concave to the T/η axis. These results suggest that the dansyl label can be covalently bound to a flexible site on succinyl-CoA synthetase and the flexibility is greatly increased on CoA binding. These results are compatible with a model for succinyl-CoA synthetase in which CoA binding induces a conformational change in the active site and help explain previous kinetic observations that the presence of CoA can affect the rates and partial reactions involving the other enzyme substrates.

Succinyl-CoA synthetase (SCS)[1] catalyzes the following reaction:

$$\text{succinate} + \text{CoA} + \text{NTP} \overset{M^{2+}}{\leftrightarrow} \text{succinyl-CoA} + \text{NDP} + P_i$$

in which NDP and NTP represent purine nucleoside di- and triphosphates, respectively. The enzyme contains two different kinds of subunits, α and β, which are present in equimolar quantities (Bridger, 1971; Brownie & Bridger, 1972). A number of functional studies have implied that protein conformational changes play a role in this reaction, but the nature of these changes has not been specified.

It appears that the active site is located at the points of contact between the α and β subunits. Thus, the α subunit contains a histidine residue that is phosphorylated during catalysis (Bridger, 1971; Brownie & Bridger, 1972). A second histidine residue of undetermined subunit location also may be involved in the catalytic reaction (Collier & Nishimura, 1979). Since the isolated α subunit (Pearson & Bridger, 1975a) is capable of being phosphorylated by ATP, it is reasonable to conclude that the nucleoside di- and triphosphate binding site, or part of it, is located on the α subunit (Pearson & Bridger, 1975b). Evidence consistent with participation of both subunits in the composition of this site has been presented recently (Ball & Nishimura, 1980). Indeed, Pearson &

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[1] Abbreviations: CoA, coenzyme A; SCS, succinyl-CoA synthetase; $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol; o-$CoAS_2$, oxidized CoA disulfide; DSCoA, desulfo-CoA; dansyl, 8-(dimethylamino)-1-naphthalenesulfonate.

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Bridger (1975b) have demonstrated a dramatic enhancement of ATP phosphorylation of the isolated α subunit when the β subunit is added. The β subunit contains the CoA binding site, as evidenced by its covalent reaction with the CoA affinity analogues oxidized CoA disulfide, o-$CoAS_2$ (Collier & Nishimura, 1978), and *S*-(4-bromo-2,3-dioxobutyl)CoA (Nishimura et al., 1980). In all likelihood, the β subunit also contains the binding site for succinate (Benson et al., 1969). Thus, it is clear that SCS catalyzes a complex reaction in which at least two protein domains provide binding and catalytic components.

There is also a body of evidence to suggest that the binding of ligands to certain sites affects the reactivity of other sites in SCS. Thus, Bridger et al. (1968) have observed that ATP greatly stimulates the rate of the succinate ↔ succinyl-CoA exchange reaction. Quite recently, Bild et al. (1980) have presented evidence that ATP binding to one active site may modulate activity at the other active site of the *Escherichia coli* enzyme. Earlier work (Grinnell & Nishimura, 1969b; Hildebrand & Spector, 1969) showed that desulfo-CoA, a competitive inhibitor of SCS but unreactive as substrate, stimulated formation of succinyl phosphate by the enzyme from ATP and succinate and the synthesis of ATP from succinyl phosphate and ADP. Each of these phenomena might be rationalized as being due to conformational changes brought about in the enzyme by the binding of ligands which are necessary for optimal catalysis to occur.

In light of the above, it is interesting that no detailed detection of any substrate-induced conformational change has been reported. We have begun to investigate this problem by making use of the sensitivity and dynamic range of fluorescence methods.

In pursuing an examination on the effects of CoA binding in terms of bringing about conformational changes in the structure of SCS, we have prepared *E. coli* enzyme that is labeled with dansyl chloride. Fluorescence polarization experiments have been carried out on this modified enzyme in the presence of various ligands. The results of this work are reported in this paper.

Experimental Procedures

All chemicals were reagent grade. [^{3}H]Dansyl chloride was a product of New England Nuclear. Dansyl chloride was from Pierce Chemical Co. Coenzyme A (lithium salt) was from P-L Biochemicals. ACA 44 was from LKB.

Purification and Assay of Succinyl-CoA Synthetase. The enzyme was purified from *E. coli* as previously described (Bowman & Nishimura, 1975). Enzyme activity was assayed either by the method of Cha (1969) or by the hydroxamate assay (Kaufman et al., 1953) as modified by Grinnell & Nishimura (1969a). The enzyme as prepared here had a specific activity of 590 units/mg (Kaufman et al., 1953).

Protein concentrations were calculated from the absorbance at 280 nm, by using an extinction coefficient, $E^{1\%}_{280nm}$, of 5.11 (Brownie & Bridger, 1972; Ramaley et al., 1967), and by the Lowry method (Lowry et al., 1951) (using succinyl-CoA synthetase as a standard).

Preparation of CoA Derivatives. The compounds oxidized CoA disulfide (o-$CoAS_2$) and desulfo-CoA were prepared as previously described (Collier & Nishimura, 1978).

Preparation of Dansyl Conjugates. SCS was modified with o-$CoAS_2$ according to the method of Collier & Nishimura (1978). The CoA-modified SCS in 100 mM sodium borate, pH 8.5, was reacted with a 2-fold molar excess of either nonradioactive or ^{3}H-labeled dansyl chloride in 5% acteone at 0 °C. After 10 min of incubation, the reaction was stopped by the addition of a 100-fold excess of hydroxylamine, and the enzyme was dialyzed against 2 L of 100 mM sodium phosphate, pH 7.5, overnight. The dialyzed enzyme was reduced with a 200-fold molar excess of DTT at 37 °C for 1 h to remove the blocking CoA group. The reduced dansylated enzyme was dialyzed against 2 L of 100 mM phosphate, pH 7.5, overnight in the cold with a buffer change after 6 h. This preparation was chromatographed on an ACA 44 gel filtration column (2 × 58 cm) equilibrated with 100 mM sodium phosphate and 500 mM KCl, pH 7.5. Fractions of 1 mL were collected at a flow rate of 10 mL/h. Fractions having SCS activity were pooled and concentrated on an Amicon PM-10 membrane.

The dansyl protein conjugate at this stage was found to be free of any free dansyl chloride by chromatography on Whatman 3MM paper, using the solvent system ethanol–100 mM sodium acetate, pH 4.5 (55:45 v/v).

The number of dansyl groups bound per mole of SCS was determined by measuring radioactivity as well as the absorption at 340 nm, using a molar extinction coefficient of 3.4×10^3 (Chen, 1968). This preparation consistently yielded a conjugate having at least 90% of the activity of the parent enzyme (535 units/mg). The enzyme was partially inactivated by high levels of dansyl incorporation (>1 mol of label/mol of enzyme), and only the most active conjugates were used in the studies reported here. The conjugates used here typically contained a dansyl:SCS ratio of 0.05 mol/mol of enzyme unless otherwise stated.

Radioactivity Measurements. Aqueous samples, up to 1 mL, were mixed with 10 mL of a solution containing 10% Biosolv and 0.7% 2,5-diphenyloxazole in toluene. Radioactivity was extracted from polyacrylamide gels by incubation of gel slices overnight at 55–60 °C in tightly stoppered scintillation vials containing 1 mL of 95% Protosol. Samples were then treated with 0.1 mL of 30% H_2O_2 for 30 min at 55–60 °C. Ten milliliters of a solution containing 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene was added for counting. All radioactivity measurements were made in a Beckman LS230 scintillation counter.

Subunit Localization of the Dansyl Binding Site. SCS labeled with [^{3}H]dansyl chloride was subjected to NaDodSO_4–polyacrylamide gel electrophoresis following the method of Bridger (1971). After electrophoresis, the subunits were located by their fluorescence and sliced, using a gel slicer, and radioactivity was measured as described earlier. Duplicate gels were stained for protein to confirm the identification on the basis of fluorescence measurements.

Fluorescence Measurements. All fluorescence measurements were made on an SLM Model 4800 spectrophotometer (SLM Instruments, Urbana, IL). Fluorescence excitation was at 350 nm, and emission was viewed through a combination of KV 389 and KV 418 filters (Schott Optical Glass, Inc., Duryea, PA). The nature of the samples used for the polarization measurements is given below (see Results).

The fluorescence polarization, P, was analyzed by the Perrin–Weber equation (Weber, 1952):

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{3\tau}{\rho_h}\right) \qquad (1)$$

Here, P_0 is the limiting polarization observed in the absence of fluorophore rotation or energy transfer, τ is the lifetime of the excited state, and ρ_h is the rotational relaxation time of the kinetic unit monitored by the probe. The reciprocal ρ_h is directly proportional to T/η, where η is the viscosity of the solution at the temperature T in kelvin. A plot of $1/P$ vs. T/η

is predicted to give a straight line with an intercept equal to $1/P_0$. In the experiments here, the ratio T/η was varied by changing the solution temperature by means of a constant-temperature water bath circulating through the cell holder of the fluorometer, and corrections were made for the temperature dependence of the viscosity where appropriate. Addition of sucrose or glycerol at concentrations between 0 and 40% (w/w) to solutions of the dansyl conjugate led to small decreases in polarization rather than the increase expected if these reagents only changed the viscosity. Therefore, measurements at constant temperature of the viscosity dependence of the polarization were not performed for the present study. The rotational relaxation time, ρ_h, can be calculated from the slope of the straight line. In cases where the Perrin–Weber plot was significantly curved, ρ_h was calculated directly from eq 1, by using a value of P_0 derived from extrapolation of the initial portion of a plot of $1/P - {}^1/_3$ vs. T/η.

Fluorescence lifetimes were measured at 20 °C on the SLM fluorometer in the phase-modulation cross-correlation configuration, using a modulating frequency of 18 MHz.

High-Performance Liquid Chromatography (HPLC). Gel filtration chromatography was performed with HPLC as well as with the ACA 44 column described above. In each case, the chromatographic behavior of the system was calibrated by reference to a series of standard proteins of known Stokes radius. HPLC was performed on an Altex Model 210 liquid chromatography apparatus, using a 60-cm TSK 3000 molecular exclusion column, and was operated at 1 mL/min. The effluent was monitored at 280 nm. Effluent fractions were collected so that enzyme activities could be monitored in addition to absorbance.

Circular Dichroism. Measurements were made with a Jasco V-500 C recording spectropolarimeter fitted with a Model DP-500 data processing unit at a temperature of 22 °C at protein concentrations ranging from 0.1 to 4 mg/mL in 100 mM sodium phosphate, pH 7.5. Cells with path lengths ranging from 0.01 to 0.1 cm were used.

Results

The native fluorescence of SCS has too short a lifetime to be used in fluorescence depolarization studies. Therefore, a conjugate of the protein with dansyl chloride was prepared. The choice of dansyl group was based on the fact that dansyl conjugates have a lifetime of approximately 12 ns, which is of the right order of magnitude to investigate rotational relaxation parameters for proteins in the molecular weight range of 10000–200000 (Weber, 1952, 1953).

Initial attempts to dansylate the protein resulted in a rapid inactivation of the enzyme. Subsequently, the active-site sulfhydryl group was protected, using the affinity label o-$CoAS_2$ (Collier & Nishimura, 1979), and then dansylated as described above under Experimental Procedures. The resulting dansyl conjugate, after removal of the active-site protecting groups, was found to have 90–95% of the original activity. The dansylated SCS was chromatographed on an ACA 44 gel filtration column (100 mM sodium phosphate and 500 mM KCl, pH 7.5) and eluted at the same volume as the native enzyme, indicating that there was no change in the native quaternary structure as a result of dansylation. $NaDodSO_4$ gel electrophoresis of the dansyl-SCS showed that both the α and β subunits were dansylated to a similar extent. Radioactivity measurements of gel slices containing α and β subunits show the ratio of α to β incorporation of [^{3}H]dansyl was equal to 1.1. This labeling ratio was independent of the total dansyl incorporation over a range of 0.05–1.3 mol of dansyl per mol of SCS tetramer.

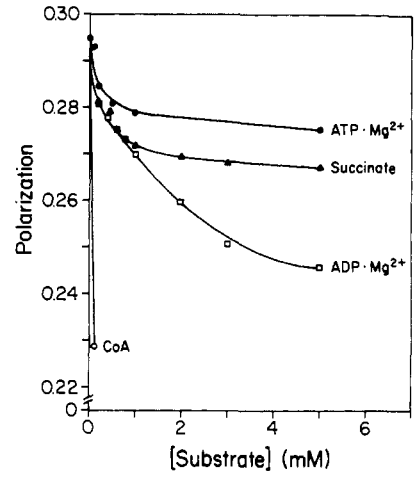


FIGURE 1: Fluorescence polarization of dansyl-SCS as a function of the concentrations of various substrates. Dansyl-SCS conjugate concentration was 0.1 mg/mL in 100 mM sodium phosphate, pH 7.5. Dansyl:SCS ratio was 0.05 mol/mol of enzyme. Temperature was maintained at 20 °C. Measurement conditions are as described under Experimental Procedures.

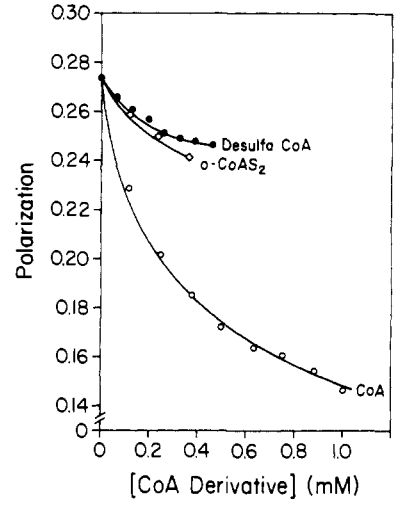


FIGURE 2: Fluorescence polarization of dansyl-SCS as a function of the concentration of CoA and CoA analogues. Solution and measurement conditions were the same as in Figure 1.

The influence of several added substrates on the fluorescence depolarization of dansyl-SCS is shown in Figure 1 for a solution containing 100 μg of dansyl-SCS per mL in 100 mM sodium phosphate, pH 7.5. Addition of the substrate ATP·Mg^{2+}, succinate, and ADP·Mg^{2+} gave a reproducible, but relatively small, drop in the measured polarization. The individual additions of either ATP or $MgCl_2$ at 5 mM gave no observable change in the polarization. On the other hand, the addition of the substrate CoA caused a large concentration-dependent drop in the fluorescence polarization. This CoA-associated decrease in the polarization was not accompanied by a significant change in the emission spectrum of the dansyl moiety. Thus, at 0.5 mM CoA, which gives most of the polarization change (Figure 2), there is no change in the dansyl emission maximum and only a slight decrease in fluorescence intensity (<10%) compatible with the change observed in the dansyl fluorescence lifetime. To test whether this change was due to the thiol character of the added CoA, we measured the polarization in the presence of either 1 mM dithiothreitol or 1 mM β-mercaptoethanol. There was no observable change in the polarization due to either mercaptan. Furthermore, addition of these thiol-containing compounds to solutions containing ATP·Mg^{2+}, ADP·Mg^{2+}, or succinate

Table I: Equilibrium Dialysis of [^{3}H]Dansyl-SCS in the Presence of CoA[a]

cell		content	dansyl groups present (nmol)
A	upper	[^{3}H]dansyl-SCS	0.310
	lower	buffer	0.026
B	upper	[^{3}H]dansyl-SCS + 4.86 mM CoA + 10 mM DTT	0.325
	lower	buffer	0.012
C	upper	[^{3}H]dansyl-SCS + 4.86 mM CoA + 10 mM DTT	0.328
	lower	buffer + 4.86 mM CoA + 10 mM DTT	0.011

[a] [^{3}H]Dansyl-SCS concentration was at 0.1 mg/mL in 100 mM phosphate, pH 7.5, buffer. Dialysis was done in cold overnight. Polarization and radioactivity measurements were done on the dialyzates.

led to no further change in the observed polarization.

Figure 2 shows the effect of concentrations of CoA between 0 and 1 mM on the fluorescence polarization of dansyl-SCS. Addition of the CoA affinity analogue o-CoAS$_2$, or desulfo-CoA, a competitive inhibitor of SCS, also gives a decrease in this polarization, although to a significantly smaller degree. Extrapolation of a double-reciprocal plot of the data in Figure 2 for CoA gives a limiting value for the polarization at high CoA concentration equal to ~0.100. Figure 2 itself then gives a value of 0.30 mM for the CoA concentration, giving half the maximum polarization effect.

To test the reversibility of the CoA effect, we treated a sample of dansyl-SCS with 0.5 mM CoA, and the polarization was measured as in Figure 2. This sample was then chromatographed on an ACA 44 column to remove bound CoA. The polarization returned to 0.269 which compared closely with that of an untreated sample. When CoA was again added to 0.6 mM, the polarization again fell and reached a value of 0.188.

The specificity of the effect of CoA was tested by the addition of CoA to samples of dansylated lactate dehydrogenase or dansylated bovine serum albumin. There was no significant change in the polarization of either conjugate at concentrations of CoA up to 5 mM.

Several alternative explanations could be given for the observed decrease in the fluorescence polarization of dansyl-SCS. The first possibility was the presence of trace amounts of noncovalently bound dansyl fluorophore which could be released upon addition of CoA. Paper chromatography of the SCS conjugate (see Experimental Procedures) showed no free label. An equilibrium dialysis experiment was done to further test for release of dye. [^{3}H]Dansyl-SCS was treated with excess CoA and then dialyzed against buffer, either with or without CoA. The results are shown in Table I. There was no significant release of any bound dansyl groups, and the samples containing CoA showed the expected low values of fluorescence polarization. Only 4–7% of the counts were detected in the buffer chamber, and more than 90% of the total counts used were recovered, thus eliminating membrane binding as an important effect.

From eq 1, it follows that any changes in the fluorescence lifetime and/or a change in the molecular size of the protein will result in a change in the observed polarization. Fluorescence lifetimes of the dansylated enzyme in the presence of two different concentrations of CoA were measured, and the results are given in Table II. There was a decrease in the fluorescence lifetime with the addition of CoA. If we assume that there was no change in the molecular size of the

Table II: Fluorescence Lifetime of Dansyl-SCS in the Presence of CoA[a]

[CoA] (mM)	lifetime (ns)
0	13
0.54	11.8
5.4	8.8

[a] Measurements were done as described under Experimental Procedures. Dansyl-SCS was at a concentration of 0.5 mg/mL in 100 mM phosphate, pH 7.5. Lifetimes are the average values from phase and modulation measurements.

protein species involved, then a decrease in the fluorescence lifetime would have given a higher value for the observed polarization. It is of interest in this regard that at 0.54 mM CoA more than 70% of the total polarization drop has occurred while the lifetime has changed by less than 10% of its initial value. In any event, as the observed polarization is smaller, there must be a change in the molelcular species involved. To test the possibility that a CoA-induced dissociation could contribute to the observed decrease in the polarization, we treated dansyl-SCS with 1 mM CoA and then subjected it to gel filtration on the ACA 44 column equilibrated with 100 mM phosphate, 0.5 M KCl, 0.2 mM CoA, and 0.4 mM DTT, pH 7.5. There was no change in the elution pattern as compared to that of either dansyl-SCS without added CoA or native unlabeled SCS. In each of these cases, SCS (M_r 140 000) eluted from the column at a volume that was the same within less than 1% of the elution volume of lactate dehydrogenase (M_r 140 000), which has approximately the same molecular weight as the $\alpha_2\beta_2$ tetramer of SCS. Gel filtration chromatography was also performed with HPLC, using a TSK 3000 column equilibrated with 50 mM sodium phosphate and 200 mM sodium sulfate, pH 7.4. In this system, there was no change in the elution profile of SCS at all CoA concentrations tested (0–1 mM).

To further test the possibility that a change in the quaternary structure of SCS could contribute to the polarization decrease, we performed HPLC, using a TSK 3000 gel filtration column, on samples ranging in SCS concentration from 25 μg/mL to 1 mg/mL. There was no change in the elution pattern of SCS at any concentration. In addition, the fluorescence polarization measurements for a sample of the dansyl-SCS conjugate were constant over the same concentration range used for the HPLC analysis.

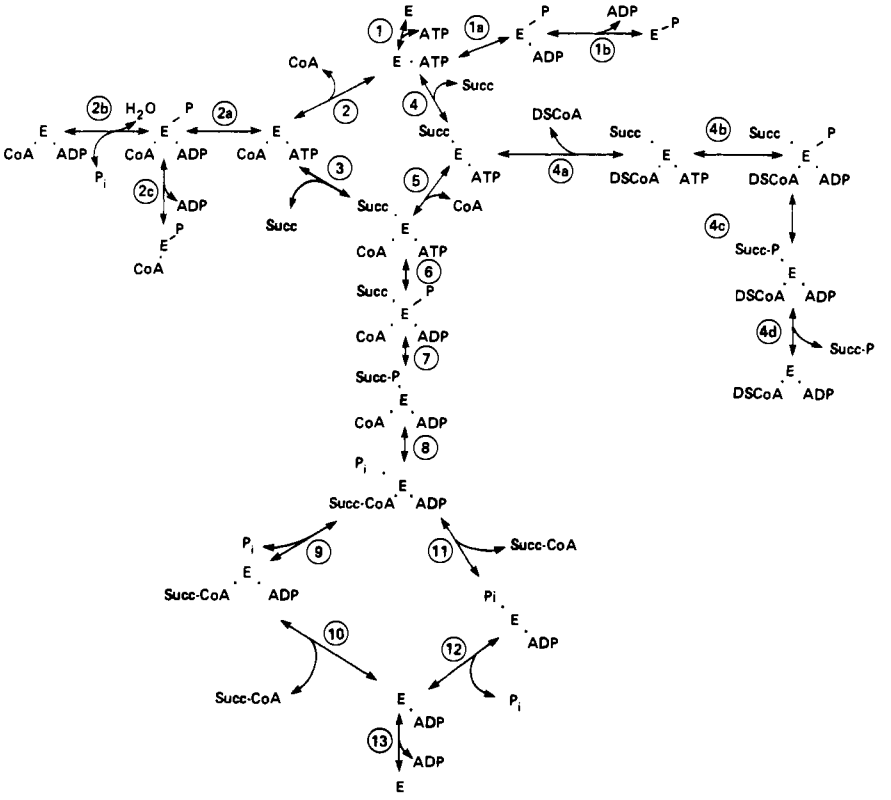
It therefore seems that the addition of CoA to dansyl-SCS brings about a conformational change in the protein which results in a greater degree of local rotational mobility of the protein segment to which the dansyl fluorophore is bound. For further investigation of this possibility, a Perrin–Weber plot was constructed for dansyl-SCS in the presence and absence of added CoA and is shown in Figure 3. Linear behavior is observed in the absence of CoA (lower curve). The data for dansyl-SCS in the presence of 0.6 mM CoA (upper curve) are not linear and display a curvature that is concave to the T/η axis. Extrapolation of the initial portion of this curve gives an intercept that is consistent with a value for P_0 that is the same as the value obtained in the absence of CoA.

Far-ultraviolet circular dichroism spectra were run in the range of 200–500 nm in the presence and absence of 1 mM CoA. The spectra were not different within the limits of experimental error and were very similar to those previously reported (Krebs & Bridger, 1974).

Discussion

The observed fluorescence depolarization of dansyl-labeled SCS in the presence of added CoA could be due to several

Scheme I



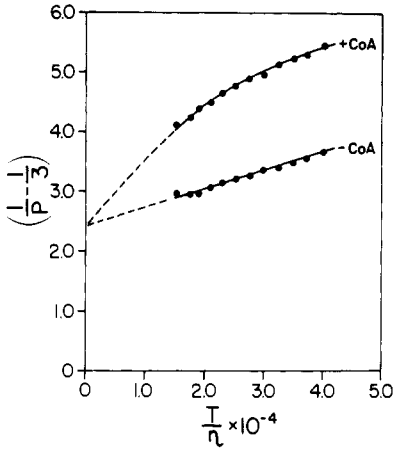


FIGURE 3: Perrin–Weber plot for dansyl-SCS in the presence and absence of CoA. Solution and measurement conditions were the same as in Figure 1. (Lower curve) Measurements in the absence of added CoA; (upper curve) measurements in the presence of 0.6 mM CoA. The T/η ratio was varied by changing the temperature.

factors, such as changes in the size of the protein (Reinhart & Lardy, 1980) or an increase in the lifetime of the excited state or the presence of free dye. None of these factors was affected with the addition of CoA. This led us to believe that there is a CoA-induced flexibility in SCS.

From the Perrin–Weber plot in Figure 3, we can get an estimate of the rotational relaxation time, $\rho_h^{20°C}$, of the dansyl conjugate of SCS. It gave a value of 105 ns ($\rho_h/\rho_0 = 0.84$) for dansyl-SCS, where ρ_0 is the rotational relaxation time calculated for SCS if it were a rigid sphere. In general, the ratio ρ_h/ρ_0 is expected to be ≥ 1 unless there is flexibility that can influence the site of labeling. Dansyl lactate dehydrogenase, which has the same molecular weight as SCS, has a $\rho_h^{25°C}$ of 188 ns ($\rho_h/\rho_0 = 1.5$) (Anderson & Weber, 1966). The observed $\rho_h^{20°C}$ for dansyl-SCS was much smaller, suggesting the flexibility of the macromolecule as a whole or of nonrigid attachment of the dansyl label to SCS. Addition of CoA brings down the $\rho_h^{20°C}$ to 35 ns ($\rho_h/\rho_0 = 0.28$). This behavior is consistent with a greatly increased flexibility of SCS as a whole and of the greater flexibility of SCS segments which are carrying the dansyl label. The suggestion of flexibility is further supported by the fact that the upper curve in Figure 3 is concave to the T/η axis, which is a result expected when there is flexibility of the fluorescent label relative to the protein (Weber, 1953).

Suggestions of conformational changes in SCS brought about by the binding of substrates have come from a variety of observations. Major changes in conformation have been suggested to accompany the phosphorylation of the enzyme. The dephosphorylated form of the enzyme is more susceptible to inactivation and proteolysis (Moffett et al., 1972). Bild et al. (1980) have demonstrated a modulating effect of ATP on $O_{P_i} \leftrightarrow O_{succinate}$ exchange during net succinyl-CoA synthesis. These workers observed a decrease in the oxygen exchange rate with increasing ATP concentration. One interpretation of these data is that ATP binding at one of the two active sites of the enzyme promotes release of the product succinyl-CoA at the other site. This release may be the result of a conformational change transmitted from one $\alpha\beta$ protomer to the next caused by phosphorylation at the site of ATP binding. No effect on oxygen exchange was observed by varying the concentration of CoA from 0.37 to 96 μM. Previous results obtained in our laboratory (Bowman & Nishimura, 1975) indicate that 2 mol of CoA can be bound per mol of enzyme without the anticooperativity that has been observed for phosphorylation of the enzyme by ATP in other laboratories (Ramaley et al., 1967; Moffett et al., 1972). These results are interesting in light of the report that there is positive cooperativity in the binding of CoA (Bowman & Nishimura, 1975). However, the results reported here do not seem to depend on the tetrameric form of the enzyme since we have

observed comparable results in fluorescence depolarization experiments with the dansyl-labeled porcine heart enzyme (A. R. S. Prasad, J. S. Nishimura, and Paul M. Horowitz, unpublished results), which has only $\alpha\beta$ quaternary structure. Bild et al. (1980) have shown that $O_{P_i} \leftrightarrow O_{succinate}$ exchange catalyzed by this enzyme is not modulated by GTP. These observations lend support to the proposal that the CoA effect described in this paper is a manifestation of structural changes that are occurring in the active-site region of the enzyme molecule. A discussion of the possible conformational changes brought about by CoA binding to succinyl-CoA synthetase is facilitated by the use of Scheme I which describes the overall reaction mechanism (reactions 1–13) and most of the partial reactions catalyzed by the enzyme. Thus, CoA-stimulated ATPase activity (Grinnell & Nishimura, 1969b) and CoA-stimulated $P_i \leftrightarrow$ ATP exchange activity (Ramaley et al., 1967) are described by the reaction sequence 1, 2, 2a, and 2b. The series of reactions 1, 2, 2a, and 2c would explain CoA-stimulated E–P $\leftrightarrow$ ATP (Ramaley et al., 1967) and ADP $\leftrightarrow$ ATP (Grinell & Nishimura, 1969a) exchange reactions. Cha et al. (1967) have also reported a CoA-stimulated GDP $\leftrightarrow$ GTP exchange reaction catalyzed by the dimeric pig heart enzyme. Finally, reactions 1, 4, 4a, 4b, 4c, and 4d, occurring in the appropriate direction, would account for desulfo-CoA-stimulated ATP production from added succinyl phosphate and ADP (Hildebrand & Spector, 1969) and succinyl phosphate synthesis from ATP and succinate (Grinnell & Nishimura, 1969b). It is reasonable to assume that the effects produced by CoA and its analogue, desulfo-CoA, are due to conformational changes in the enzyme, since these compounds are not bound covalently. The recent observation of Vogel & Bridger (1982) that addition of 0.5 mM CoA caused a downfield shift and broadening of the ^{31}P NMR resonance of succinyl-CoA synthetase lends additional support to the notion that the binding of CoA brings about conformational changes in the enzyme. Perhaps, as Bridger (1981) has suggested recently, occupancy of substrate subsites may be required for the correct orientation of the catalytic machinery of the enzyme. The enzyme may be required to undergo a significant conformational change in assuming this orientation.

Equilibrium dialysis measurements of the binding of CoA have given dissociation constants for the SCS–CoA complex in the range of 0.04–0.40 mM (Bowman & Nishimura, 1975), the exact value depending on the nature of the preparation tested. The value of the CoA concentration (0.30 mM) observed to give half the maximum polarization change is compatible with the values from equilibrium dialysis determined for homogeneous enzyme with a specific activity comparable to that used here (J. S. Nishimura, unpublished results). It is of interest that the K_m of ~ 1 μM determined for the catalyzed reaction (Moffett & Bridger, 1970) is much smaller than any determination of the dissociation constant. Although the K_m cannot directly be used to assess binding, this comparison may reflect the occurrence of further conformational changes in the catalyzed reaction.

The observation that the circular dichroism spectrum is independent of the presence of CoA appears to confirm that the flexibility increase observed is a localized change in the protein structure.

Because the covalently bound dansyl fluorophore is distributed almost equally between the α and β subunits, we do not know at this time if the proposed conformational changes involve structures in only one subunit or both. In an attempt to reach conclusions in this regard, we plan to carry out experiments in which isolated dansyl-labeled and unlabeled subunits will be mixed. For this purpose, we intend to employ the methodology described by Pearson & Bridger (1975a).

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