S-Trifluoroacetonyl-Coenzyme A: A [19]F Analogue of Acetyl-Coenzyme A[†]

Kenichi K. Yabusaki[‡] and Clinton E. Ballou*

ABSTRACT: S-Trifluoroacetonyl-coenzyme A has been synthesized in 87% yield by reaction of 1,1,1-trifluoro-3-bromopropanone with trilithium coenzyme A in presence of pyridine. The compound was characterized by its ultraviolet absorption spectrum and ^{1}H and ^{19}F nuclear magnetic resonance spectra. The α-methylene protons of the S-trifluoroacetonyl group exchanged with D_2O and showed a pK_a of 9.85 in S-trifluoroacetonylmercaptoethanol. S-Trifluoroacetonyl-coenzyme A is a competitive inhibitor of porcine heart citrate synthetase ($K_i = 0.16$ mM). It forms a binary complex with the enzyme and a ternary complex with the enzyme/oxaloacetate binary complex, as evidenced by the ^{19}F shift. S-Trifluoroacetonyl-coenzyme A and S-trifluoroacetonylmercaptoethanol form weak to moderately strong complexes with α-cyclodextrin and show little or no interaction with the methylglucose polysaccharide and lipopolysaccharides from Mycobacterium smegmatis [Smith, W. L., & Ballou, C. E. (1973) J. Biol. Chem. 248, 7118]. S-Trifluoroacetonylmercaptoethanol probably forms an inclusion complex with α-cyclodextrin because the interaction is reversed by compounds that do form inclusion complexes.

A primary interest in this laboratory has been the characterization of the methylated polysaccharides in Mycobacterium smegmatis (Saier & Ballou, 1968; Smith & Ballou, 1973; Gray & Ballou, 1975) that appear to regulate the fatty acid synthetase complex of this organism (Ilton et al., 1971). These polymethylpolysaccharides have been shown to alter the fatty acid product distribution and to reduce the K_m for acetyl-coenzyme A and malonyl-coenzyme A when added to incubations of the fatty acid synthetase from M. smegmatis, apparently as a result of specific interaction with the products and the synthetase.

As a first step in a search for sensitive probes to study the mechanism of these effects, we have synthesized S-trifluoroacetonyl-coenzyme A and studied its binding by ^{19}F NMR to the 6-O-methylglucose polysaccharide of M. smegmatis and related compounds. To test the general utility of this substance as an analogue of acetyl-coenzyme A, we have also investigated its inhibition of and binding to porcine heart citrate synthetase. The results suggest that S-trifluoroacetonyl-coenzyme A can be a useful probe for studies of acyl-coenzyme A binding sites on macromolecules.

Experimental Section

Materials. 1,1,1-Trifluoro-3-bromopropanone was obtained from Peninsular Chem Research, β-mercaptoethanol and benzoic acid were from Eastman Organic Chemicals, and 5,5'-dithiobis(2-nitrobenzoic acid) was from Aldrich Chemical Co. Lithium coenzyme A, acetyl-coenzyme A, and n-hexanoyl-coenzyme A were from P-L Biochemicals. Oxaloacetic acid and tris(hydroxymethyl)aminomethane were from Sigma

Chemical Co. Porcine heart citrate synthetase, as a crystalline suspension in 2.2 M ammonium sulfate, pH 7.0, was from P-L Biochemicals (lot 646-13) and Sigma Chemical Co. (lot 66C-8600). The citrate synthetase preparations were homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Weber & Osborn, 1969), and the enzyme concentrations were determined spectrophotometrically at 280 nm using an absorbance index ($E_{1cm}^{1\%}$) of 17.8 (Singh et al., 1970).

The 6-O-methylglucose polysaccharide and the 6-O-methylglucose lipolysaccharide were isolated and purified as described previously (Saier & Ballou, 1968). α-Cyclodextrin was a gift from Dr. F. Carmer, Max-Planck Institute for Experimental Medicine, Göttingen. LH-20 Sephadex was from Pharmacia Fine Chemicals, Inc., Polygram Cel 300 precoated thin layer sheets were from Brinkmann Instruments, Inc., 99.8% deuterium oxide was from Stohler Isotope Chemicals, and sodium 3-(trimethylsilyl)propanesulfonate was from Wilmad Glass Co. Other chemicals were reagent grade.

S-Trifluoroacetonylmercaptoethanol (F_3CCOCH_2–S–CH_2CH_2OH) was prepared by the dropwise addition of 3.8 g (20 mmol) of 1,1,1-trifluoro-3-bromopropanone to 1.4 mL (20 mmol) of β-mercaptoethanol in 0.5 mL of 1 M phosphate buffer (pH 7.0) under rapid magnetic stirring at 4 °C. The reaction was kept at 4 °C and maintained at pH 7.0–8.0 by the addition of 40% potassium hydroxide. The white crystalline product that formed was collected on a Buchner funnel, washed with several volumes of water, and dried. It was further purified by recrystallization from benzene and ligroin (Huestis & Raftery, 1972) and characterized by its ^{1}H and ^{19}F NMR spectra.

Methods. Ultraviolet and visible spectra were recorded on Cary 14 and 15 spectrophotometers using 1-cm quartz cuvettes maintained at 27 ± 0.5 °C by a jacketed cuvette holder. Citrate synthetase activity was determined by measuring the initial rate of reaction at 412 nm of liberated coenzyme A with 5,5'-dithiobis(2-nitrobenzoic acid) (Srere et al., 1963). A typical reaction contained 0.1 μmol of 5,5'-dithiobis(2-nitrobenzoic acid), 0.01 μmol of potassium oxaloacetate, 100 μmol of tris(hydroxymethyl)aminomethane hydrochloride buffer (pH
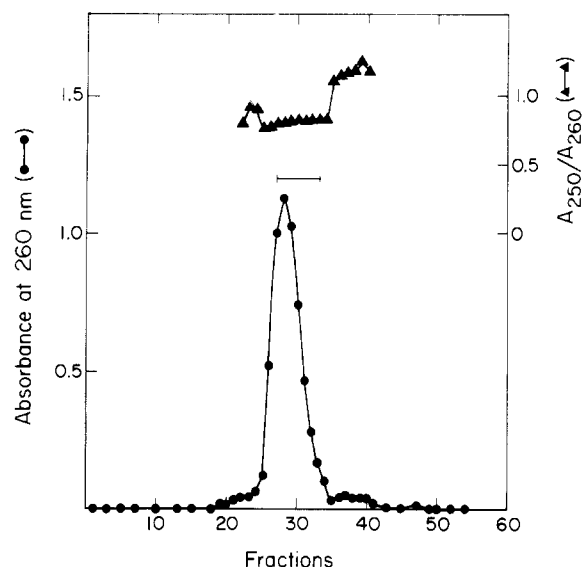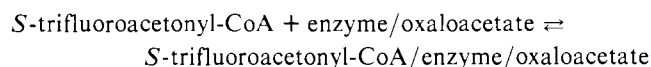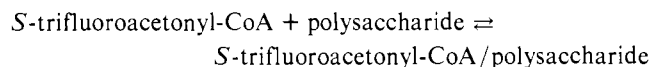
FIGURE 1: Elution profile of S-trifluoroacetonyl-coenzyme A chromatographed on LH-20 Sephadex using ethanol–water (2:3, v/v) as the solvent. The constant $A_{250/260}$ indicates an absence of contamination with pyridinium hydrobromide.
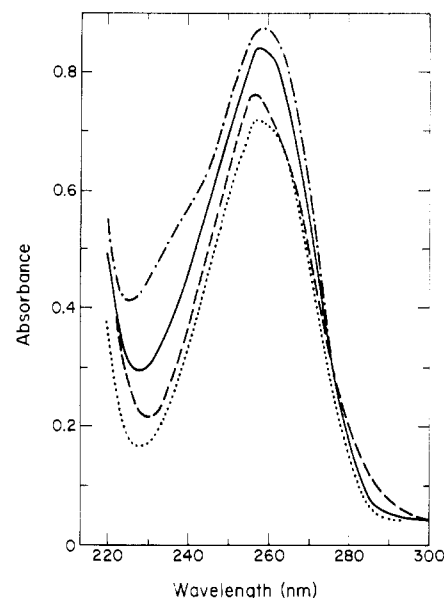

FIGURE 2: Ultraviolet absorption spectra of 0.044 mM S-trifluoroacetonyl-coenzyme A in water (···), in 0.1 N HCl (- - -), and in 0.1 N KOH (—); and of 0.054 mM acetyl-coenzyme A in water (· · · ·).

8.1), varying amounts of acetyl-coenzyme A, S-trifluoroacetonyl-coenzyme A, and enzyme solution in a total volume of 1.0 mL. The reaction, initiated by the addition of enzyme, was carried out at 27 °C. Initial velocity data were plotted according to Lineweaver–Burk (1934).

For the detection of adenine-containing compounds on thin-layer chromatograms by their ultraviolet absorption, a Chromato-vue cabinet (Ultra-Violet Products, Inc.) was used. Free sulfhydryl groups were determined by the method of Ellman (1959). Proton magnetic resonance spectra were recorded at 100 and 220 MHz on Varian XL-100-15 and HR-220 spectrometers operating in the Fourier transform and continuous wave modes, respectively. [19]F spectra were recorded at 94.1 MHz using a Varian XL-100-15 spectrometer operating in the Fourier transform mode.

The pH of phosphate buffer solutions in D_2O for NMR experiments was obtained by adding 0.4 to the measured pH (Lumry et al., 1951). An S-trifluoroacetonylmercaptoethanol solution in a capillary was used as an external reference for the ^{19}F spectra.

The binding of S-trifluoroacetonyl-coenzyme A to methylglucose polysaccharide and citrate synthetase/oxaloacetate complex by ^{19}F NMR measurements was analyzed according to Raftery et al. (1968) in terms of the following equilibria.

S-trifluoroacetonyl-CoA + polysaccharide $\rightleftharpoons$

S-trifluoroacetonyl-CoA/polysaccharide

S-trifluoroacetonyl-CoA + enzyme/oxaloacetate $\rightleftharpoons$

S-trifluoroacetonyl-CoA/enzyme/oxaloacetate

Srere (1966) has shown oxaloacetate to form a tight binary complex with citrate synthetase, with a dissociation constant of 0.6 μM in 4 M urea, and concentrations were chosen such that all of the enzyme was in the binary complex.

Titrations of S-trifluoroacetonyl-coenzyme A/methylglucose polysaccharide binding were done by transferring a fixed concentration of S-trifluoroacetonyl-coenzyme A in an NMR tube into a vial containing a known amount of solid polysaccharide, thoroughly mixing them, then carefully transferring the mixture back into the NMR tube. Initial experiments showed that the downfield shift of the ^{19}F NMR signal of S-

trifluoroacetonyl-coenzyme A in the presence of citrate synthetase was dependent on the oxaloacetate concentration. Therefore, the effect of oxaloacetate on the binary S-trifluoroacetonyl-coenzyme A/citrate synthetase complex was also investigated. Titrations were done by adding aliquots of a stock solution either of S-trifluoroacetonyl-coenzyme A or oxaloacetate or citrate synthetase/S-trifluoroacetonyl-coenzyme A binary complexes in an NMR tube using a calibrated micropipet. In all experiments, the total volume change due to the addition of either reagent was less than 2%.

Results

Preparation of S-Trifluoroacetonyl-Coenzyme A. In a nitrogen atmosphere, 0.05 mmol of trilithium coenzyme A was dissolved in 2 mL of distilled water, 0.04 mL (0.5 mmol) of pyridine was added, and the mixture was stirred magnetically. Then 0.055 mL (0.16 mmol) of 1,1,1-trifluoro-3-bromopropanone was added and the reaction was kept at room temperature (23 °C). After 20 min, the absence of free sulfhydryl group (Ellman, 1959) indicated the reaction was complete, so the mixture was evaporated to dryness under reduced pressure and the residue, dissolved in 2 mL of water, was applied to an LH-20-Sephadex column (2 × 39 cm) equilibrated at 4 °C with ethanol–water (2:3, v/v). Fractions of 2.3 mL were collected by elution with the ethanol–water solvent and assayed at 260 nm for adenine content (Figure 1). The ultraviolet absorbing material in fractions 35–41 was assumed to be pyridinium hydrobromide and was not further characterized. After combining fractions 27–33, the solvent was removed at reduced pressure and the product was dried for 48 h under vacuum over anhydrous calcium chloride at room temperature, yielding 44 mg of a fluffy white powder, 87% yield based on the dihydrate of the trilithium salt of coenzyme A. S-Trifluoroacetonyl-coenzyme A gave a single ultraviolet-absorbing spot on thin-layer cellulose chromatography using the solvent 2% aqueous acetic acid–acetonitrile (1:1, v/v) (Clements et al., 1976). The R_f values for coenzyme A, acetyl-coenzyme A, and S-trifluoroacetonyl-coenzyme A were 0.78, 0.86, and 0.85, respectively.

Ultraviolet Spectra of S-Trifluoroacetonyl-Coenzyme A. S-Trifluoroacetonyl-coenzyme A showed an absorption
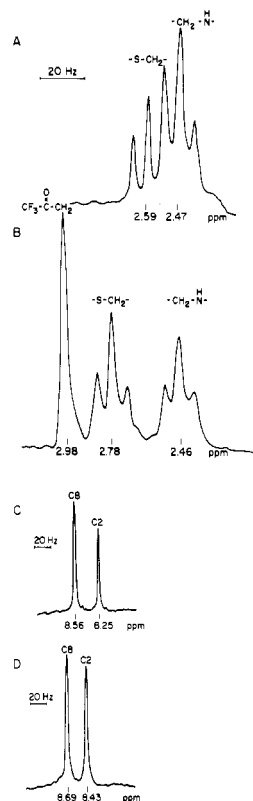
FIGURE 3: ¹H NMR 100-MHz spectra of coenzyme A and S-trifluoroacetonyl-coenzyme A. (A) The cysteamine methylene protons of 0.028 M coenzyme A. (B) The cysteamine methylene protons and the trifluoroacetonyl methylene protons of 0.03 M S-trifluoroacetonyl-coenzyme A. Note the shift of the cysteamine methylene protons due to substitution on the sulfur atom. (C) The C2 and C8 protons of the adenine ring of coenzyme A. (D) The C2 and C8 protons of the adenine ring of S-trifluoroacetonyl-coenzyme A.

maximum at 259 nm, with a spectrum very similar to coenzyme A at pH 5.6. Lowering the pH resulted in the characteristic shift of the maximum from 259 to 257 nm (Figure 2) (Morrell & Bock, 1957). Absence of a thioester bond in the compound is evident from the lack of the shoulder at 232 nm observed for acetyl-coenzyme A. The possibility of N-alkylation on the adenine ring is excluded by the absence of the characteristic absorbance bands (Singer et al., 1974).

Proton Magnetic Resonance Spectra of S-Trifluoroacetonyl-coenzyme A. The ¹H NMR spectrum of S-trifluoroacetonyl-coenzyme A was consistent with those reported for various coenzyme A derivatives (Mieyal et al., 1974, 1976; Lee & Sarma, 1974, 1975). The major differences between reduced coenzyme A and S-trifluoroacetonyl-coenzyme A are in a two-proton absorption assigned to the α-methylene protons of the trifluoroacetonyl group, 2.98 ppm downfield from external sodium 3-(trimethylsilyl)propanesulfonate; the downfield chemical shift of the cysteamine methylene protons adjacent to the sulfur atom which have been shown to be sensitive to substitutions on the sulfur for a number of coenzyme A derivatives (Figure 3) (Mieyal et al., 1974); and 0.18 and 0.13 ppm downfield shifts of the C-2 and C-8 protons of the adenine ring relative to their positions in coenzyme A (Figure 3).

We observed that the ¹H NMR signal for the α-methylene protons of the S-trifluoroacetonyl group slowly diminished with time in deuterium oxide (Table I), an obvious mechanism for this exchange being by way of keto–enol tautomerism. To confirm this possibility, the pK_a of the α-methylene protons was determined for the model compound S-trifluoroacetonylmercaptoethanol by direct measurement of the enolate

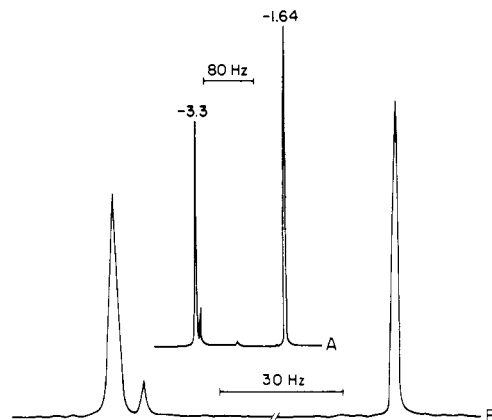FIGURE 4: ¹⁹F NMR spectra of (A) S-trifluoroacetonyl-coenzyme A (left) and S-trifluoroacetonylmercaptoethanol (right) at a sweep width of 2000 Hz, the numbers referring to the relative chemical shifts in ppm; and (B) at the decreased sweep width. The small peak to the right of the S-trifluoroaceonyl-coenzyme A signal is due to a minor impurity in the reference sample.

TABLE I: Deuterium Oxide Exchange of α-Methylene Protons of the S-Trifluoroacetonyl-Coenzyme A.

Time (h)	No. of α-methylene protons remaining in the S-trifluoroacetonyl group[a]
0	2[b]
24	1.6
168	0.8
240	0.5
330	0.3

[a] Spectra were recorded at 220 MHz on a 40 mM solution of the lithium salt in D_2O at pD 3.7. The α-methylene protons were integrated relative to the internal nonexchangeable methine proton of the pantetheine moiety of S-trifluoroacetonyl-coenzyme A. [b] By extrapolation to zero time.

ion, from its absorbance at 250–270 nm, as a function of pH.

$$\underset{F_3CCCH_2SR}{\overset{O}{\underset{\|}{}}} \rightleftharpoons \underset{F_3CC=CHSR}{\overset{O^-}{\underset{|}{}}} + H^+$$
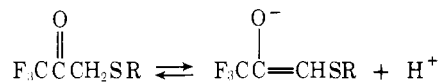
A typical titration curve was obtained which gave an apparent pK of 9.85 and agrees with a pK_a of 9.8 determined for S-trifluoroacetonyl alkyl sulfides (Bendall & Lowe, 1976).

Fluorine-19 NMR Spectrum of S-Trifluoroacetonyl-Coenzyme A. The ¹⁹F NMR spectrum of S-trifluoroacetonyl-coenzyme A in D_2O showed a singlet 1.66 ppm (156.2 Hz) downfield from the internal reference S-trifluoroacetonylmercaptoethanol (Figure 4). Assuming the molar extinction coefficient for S-trifluoroacetonyl-coenzyme A at pH 7.0 to be 15.4×10^3 M⁻¹, as reported for other coenzyme A derivatives (Kumar et al., 1970), the fluorine to adenine ratio was three in agreement with the expected value.

Inhibition of Citrate Synthetase by S-Trifluoroacetonyl-Coenzyme A. Initial velocity measurements in 10 μM oxaloacetate and 20, 40, and 60 μM S-trifluoroacetonyl-coenzyme A, with acetyl-coenzyme A as the varied substrate, showed S-trifluoroacetonyl-coenzyme A to be a competitive inhibitor of porcine heart citrate synthetase (Figure 5). From a Dixon plot, a K_i of 0.16 mM was determined, compared with a K_m of 11 μM for acetyl-coenzyme A.
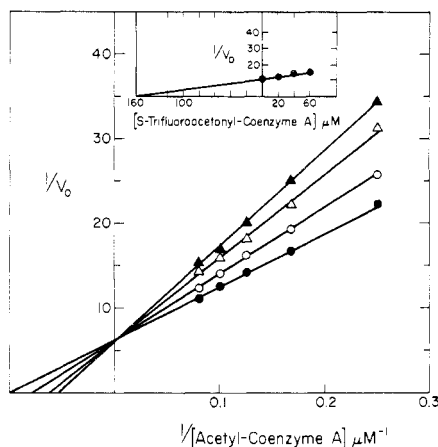
FIGURE 5: Double-reciprocal plots of initial velocities as a function of acetyl-coenzyme A concentration at various fixed concentrations of S-trifluoroacetonyl-coenzyme A and 10 μM oxaloacetate. The K_m was 11 μM for acetyl-coenzyme A and 2.5 μM for oxaloacetate. No addition (●), 20 μM (○), 40 μM (△), and 60 μM (▲) S-trifluoroacetonyl-coenzyme A. The inset is a Dixon plot at 12 μM acetyl-coenzyme A. V_0 is expressed in nmol of coenzyme A released per min per mg of enzyme protein.
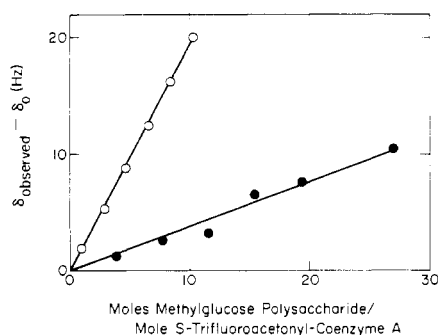

FIGURE 6: ^{19}F chemical shifts of S-trifluoroacetonyl-coenzyme A in presence of the methylglucose polysaccharide. The chemical shifts are relative to external S-trifluoroacetonylmercaptoethanol and δ_0 is the shift in Hz of free S-trifluoroacetonyl-coenzyme A. (●) is 1.2 mM, and (○) is 5 mM S-trifluoroacetonyl-coenzyme A, both in 0.1 M phosphate buffer, pH 7.0.

NMR Studies of S-Trifluoroacetonyl-Coenzyme A and S-Trifluoroacetonylmercaptoethanol with Methylglucose Polysaccharide, Methylglucose Lipopolysaccharide, and α-Cyclodextrin. The ^{19}F NMR spectra of 1.2 and 5 mM S-trifluoroacetonyl-coenzyme A, in 0.1 M phosphate buffer at pH 7.0, were recorded in the presence of increasing concentrations of methylglucose polysaccharide (Figure 6). Even at a ratio of 27 mol of methylglucose polysaccharide to 1 mol of S-trifluoroacetonyl-coenzyme A, the binding does not saturate. Addition of acetyl-coenzyme A or n-hexanoyl-coenzyme A in five times the amount of the S-trifluoroacetonyl-coenzyme A did not reverse the ^{19}F shift, suggesting a lack of competition in the binding. Similar results were obtained for S-trifluoroacetonyl-coenzyme A in the presence of methylglucose lipopolysaccharide.

The ^{19}F resonance of 2 mM S-trifluoroacetonylmercaptoethanol was shifted about 50 Hz by 36 mM α-cyclodextrin (Table II). This could be due to the formation of an inclusion complex, and this is supported by the observation that benzoic acid reverses the ^{19}F shift, presumably because it displaces the S-trifluoroacetonylmercaptoethanol (Schlenk & Sand, 1961). In contrast, the ^{19}F resonance of 2 mM S-trifluoroacetonylmercaptoethanol was affected very little by 10 mM methylglucose lipopolysaccharide. If the magnitude of the chemical

TABLE II: Fluorine-19 Chemical Shifts of S-Trifluoroacetonyl-Coenzyme A and S-Trifluoroacetonylmercaptoethanol in the Presence of Polysaccharide Ligands.

Derivative	Ligand	
	Methylglucose lipopolysaccharide (Hz)	α-Cyclo-dextrin
S-Trifluoroacetonyl-coenzyme A[a]	−5[b]	−3.8[c]
		−8.4[d]
S-Trifluoroacetonyl-mercaptoethanol[a]	−1.9[b]	−31[c]
		−50[d]
S-Trifluoroacetonyl-mercaptoethanol[e]		−47[f]
		−45[g]
		−34[h]

[a] Spectra were recorded at ambient probe temperature with 2 mM S-trifluoroacetonyl derivative in D_2O. Chemical shifts are relative to the signal position in the absence of ligand. [b] In the presence of 10 mM methylglucose lipopolysaccharide II. [c] In the presence of 16 mM α-cyclodextrin. [d] In the presence of 36 mM α-cyclodextrin. [e] Spectra were recorded at ambient probe temperature with 2 mM S-trifluoroacetonylmercaptoethanol in D_2O. Chemical shifts are relative to signal position in absence of ligand referenced to external trifluoroacetic acid. [f] In the presence of 36 mM α-cyclodextrin. [g] In the presence of 36 mM α-cyclodextrin and 4.7 mM acetyl-coenzyme A. [h] In the presence of 36 mM α-cyclodextrin and 2.4 mM benzoic acid.

shift for the trifluoroacetonyl group is taken as a criterion of binding, it appears that the polysaccharide binds neither trifluoroacetonyl derivative very well and that the cyclodextrin forms a good complex only with the mercaptoethanol derivative. Apparently the coenzyme A moiety interferes with the formation of an inclusion complex. Acetyl-coenzyme A did not displace S-trifluoroacetonylmercaptoethanol from its complex with cyclodextrin.

NMR Studies of S-Trifluoroacetonyl-Coenzyme A with Porcine Heart Citrate Synthetase. Owing to the relative insensitivity of the NMR technique, the following measurements were done with mM concentrations of reactants. All experiments were done in 0.1 M phosphate at pH 7.0. The ^{19}F NMR signal for 2.2 mM S-trifluoroacetonyl-coenzyme A, in presence of 2.2 mM potassium oxaloacetate, absorbs 161 Hz downfield of external S-trifluoroacetonylmercaptoethanol (Figure 7A). On the other hand, the ^{19}F NMR signal for 2.5 mM S-trifluoroacetonyl-coenzyme A, in presence of 0.6 mM citrate synthetase, is shifted 17 Hz downfield from free S-trifluoroacetonyl-coenzyme A, with a slight broadening of the ^{19}F resonance (Figure 7B). The addition to this mixture of oxaloacetate to a final concentration of 2.5 mM results in a 40-Hz downfield shift with further broadening of the ^{19}F NMR resonance (Figure 7C). Addition of more oxaloacetate did not appreciably shift or broaden the signal.

Accurate quantitation of the observed resonance lines in terms of T_2 relaxation times is difficult unless the sample homogeneity is carefully controlled for each experiment. However, the results in Figure 7 clearly demonstrate a qualitative effect of citrate synthetase and oxaloacetate on the ^{19}F NMR signal for S-trifluoroacetonyl-coenzyme A. When additional S-trifluoroacetonyl-coenzyme A was added to a solution of 2.5 mM S-trifluoroacetonyl-coenzyme A, 2.5 mM oxaloacetate, and 0.6 mM citrate synthetase, to final concentrations of 5.0 and 7.5 mM, the line widths of the ^{19}F NMR signals decreased and upfield shifts occurred of 16 and 24 Hz, respectively (Table III). Since we assume conditions of rapid chemical exchange where the observed ^{19}F NMR resonance line represents a weighted average between the free and various bound states,
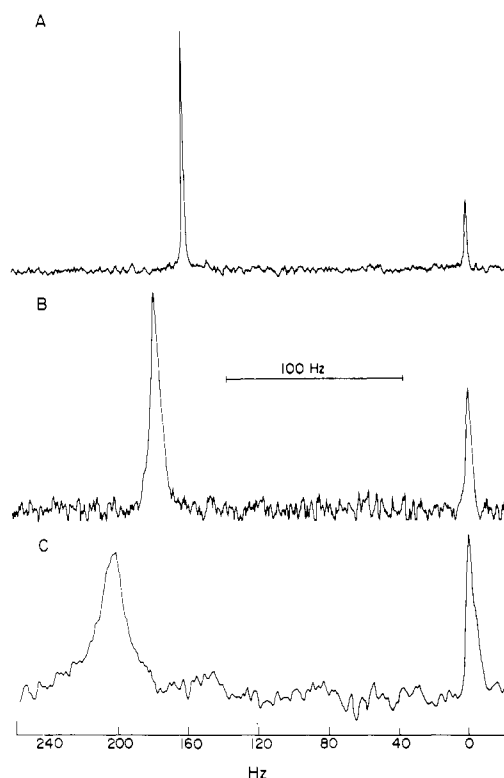
FIGURE 7: ^{19}F NMR spectra. (A) S-Trifluoroacetonyl-coenzyme A, 2.2 mM, plus 2.2 mM oxaloacetate. (B) S-Trifluoroacetonyl-coenzyme A, plus 0.6 mM citrate synthetase. (C) S-Trifluoroacetonyl-coenzyme A, 2.5 mM, plus 0.6 mM citrate synthetase and 2.5 mM oxaloacetate. The signal at Hz = 0 is external S-trifluoroacetonylmercaptoethanol.

FIGURE 8: Titration of binary S-trifluoroacetonyl-coenzyme A/citrate synthetase with potassium oxaloacetate. The S-trifluoroacetonyl-coenzyme A was 2.5 mM and the citrate synthetase was 0.6 mM. δ_0 is the chemical shift of 2.2 mM S-trifluoroacetonyl-coenzyme A plus 2.2 mM oxaloacetate. All measurements were done in 0.1 M phosphate buffer at pH 7.0.

TABLE III: ^{19}F Chemical Shifts of S-Trifluoroacetonyl-Coenzyme A in the S-Trifluoroacetonyl-Coenzyme A/Citrate Synthetase/Oxaloacetate Complexes.

Ligand and concn (mM)			^{19}F NMR data		
S-Trifluoro-acetonyl-co-enzyme A	Oxalo-acetate	En-zyme	Obsd[a] chem shifts (Hz)	δ (Hz)	$1/\delta$ (s)
2.2	2.2	0	161	0	
2.5	0	0.6	178	17	0.06
2.5	2.5	0.6	208	47	0.02
5.0	2.5	0.6	192	31	0.03
7.5	2.5	0.6	184	23	0.04

[a] Relative to external S-trifluoroacetonylmercaptoethanol.

these changes can be interpreted as resulting from an increased concentration of the free S-trifluoroacetonyl-coenzyme A species. Alternatively, the results could reflect interaction of S-trifluoroacetonyl-coenzyme A at secondary sites on the protein which affect both the relaxation rates and local electronic environments of the ^{19}F nuclei. The data in Table III, plotted according to Raftery et al. (1968), yield an apparent dissociation constant for the ternary S-trifluoroacetonyl-coenzyme A/citrate synthetase/oxaloacetate complex of 1.4 mM and a limiting chemical shift of the S-trifluoroacetonyl-coenzyme A for the fully bound species of 3.85 ppm. Because the concentrations of the various S-trifluoroacetonyl-coenzyme A/citrate synthetase/oxaloacetate complexes are related to each other by the ratio of their association constants (Raftery et al., 1968), it was not possible to establish the formation of a 1:1:1 enzyme/oxaloacetate/S-trifluoroacetonyl-coenzyme A ternary complex. Weidmann et al. (1973) showed that the presence of oxaloacetate did not affect the affinity of a spin-label analogue of acetyl-coenzyme A for porcine heart citrate synthetase. From the above dissociation constant, we calculate that at 2.5 mM S-trifluoroacetonyl-coenzyme A and 0.6 mM citrate synthetase the enzyme is all in the form of the binary complex. Therefore, to a first approximation the dissociation constant is assumed to be the same for the binary S-trifluoroacetonyl-coenzyme A/enzyme and ternary S-trifluoroacetonyl-coenzyme A/enzyme/oxaloacetate complexes.

Titration of the binary complex (2.5 mM S-trifluoroacetonyl-coenzyme A in the presence of 0.6 mM citrate synthetase) with oxaloacetate resulted in both a downfield shift and broadening of the ^{19}F NMR signal, with saturation occurring at 1.5 mol of oxaloacetate per mol of enzyme (Figure 8). However, because of the high concentration of oxaloacetate used for the NMR measurements, the observed downfield
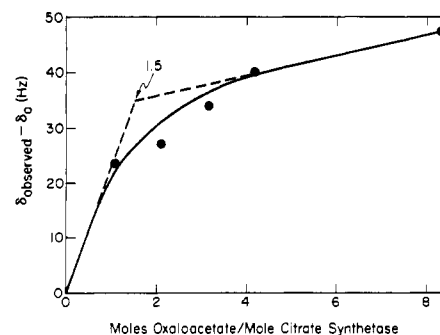
shifts and line broadening of the ^{19}F signals at levels above 1–2 mol of oxaloacetate per mol of citrate synthetase may represent nonspecific effects on the trifluoroacetonyl group due to binding of oxaloacetate at secondary sites.

Discussion

In this study, we have been concerned with the synthesis and potential applications of S-trifluoroacetonyl-coenzyme A for investigating acetyl-coenzyme A binding sites on macromolecules by ^{19}F NMR techniques. The ready synthesis of S-trifluoroacetonyl-coenzyme A by alkylation of coenzyme A with 1,1,1-trifluoro-3-bromopropanone is consistent with the recognized nucleophilicity of the coenzyme A sulfhydryl group.

$$CF_3COCH_2Br + HS\text{-coenzyme A}$$
$$\rightarrow CF_3COCH_2\text{-S-coenzyme A} + HBr$$
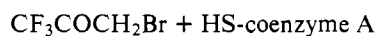
The ^{1}H and ^{19}F NMR and the ultraviolet spectral data support the proposed structure. In addition, we present evidence that the S-trifluoroacetonyl group undergoes proton exchange (Table I), with an apparent pK of 9.85 for the α-methylene protons in the mercaptoethanol derivative. The slow rate of exchange of the methylene protons is not inconsistent with their acid strength since, as discussed by Gould (1959), the correlation between these two factors is not always good. Thus, in water at 25 °C, the methyl protons of nitromethane (pK_a = 10.2) exchange with a half-time of 2.7 × 10^5 min, whereas the methylene protons of ethyl acetoacetate (pK_a = 10.7) exchange with a half-time of 9.6 min (Pearson & Dillon, 1953). Trifluoromethyl ketones are also known to show rates of ex-

change that are slower than might be expected from their acid strengths. The half-time for methylene proton exchange in S-trifluoroacetonylmercaptoethanol is about 10^4 min, and this is not too far from that extrapolated from the data for trifluoromethyl ketones in Figure 1 of the paper by Pearson & Dillon (1953).

The reactivity of the SH group of coenzyme A to alkylation (Lynen et al., 1951; Friedmann et al., 1952; Gregory, 1955) is well substantiated. In attempting to synthesize 2-bromoacetyl-coenzyme A by reaction of coenzyme A with 2-bromoacetylthiophenol under conditions first described by Chase & Tubbs (1969), Clements et al. (1976) obtained phenylthiocarboxymethyl-coenzyme A as the major product. Moreover, analogues of coenzyme A containing the thioester bond are kinetically more reactive than corresponding oxygen esters as demonstrated by the extreme lability of the thioester in 2-bromoacetyl-coenzyme A (Clements et al., 1976).

[19]F chemical shifts are dominated by p-orbital electrons (Emsley & Phillips, 1971), and the ^{19}F nucleus has several advantages over the proton as an NMR probe (Dwek, 1973; Huestis & Raftery, 1972; Bode et al., 1975a,b; Longmuir & Dahlquist, 1976). An interesting feature of the ^{19}F NMR spectrum of S-trifluoroacetonyl-coenzyme A is that it absorbs 1.66 ppm (156.2 Hz) downfield from S-trifluoroacetonylmercaptoethanol. Since the S-trifluoroacetonyl group is bonded identically with a sulfur atom in both derivatives, the chemical shift differences can be ascribed largely to diamagnetic and paramagnetic contributions from neighboring nuclei. The observed chemical shifts can arise from magnetic anisotropies caused by aromatic groups, van der Waals interactions, electric field effects from charged groups, and specific bonding interactions (Buckingham et al., 1960; Huestis & Raftery, 1972; Millet & Raftery, 1972; Dwek, 1973; Bode et al., 1975a,b). Giessner-Prette & Pullman (1971) estimate that aromatic ring currents can lead to shifts of 100–200 Hz. Petrukis & Bernstein (1963) have shown that the ^{19}F chemical shift in HCF_3 is 15 times more sensitive to van der Waals perturbation than the proton chemical shift in the same molecule. However, one would not expect chemical shifts caused by van der Waals interactions to be as large as those caused by magnetic anisotropies. Since the S-trifluoroacetonyl group is neutral, we do not expect charge interactions to be important. Finally, because the electrons about the fluorine atoms are tightly held, specific hydrogen bonding should be minimal (Longmuir & Dahlquist, 1976). Thus, the most reasonable explanation for the 1.66 ppm downfield shift would be a combination of intramolecular van der Waals interactions and ring current effects. For such effects to occur, the coenzyme A would probably have to assume a folded conformation bringing the S-trifluoroacetonyl group into proximity with the adenine ring.

Support for this supposition comes from recent ^{1}H NMR studies on the solution conformations of various coenzyme A derivatives (Mieyal et al., 1974, 1976; Lee & Sarma, 1974, 1975; Wilson et al., 1975). More recently, Perahia & Cebe (1977) analyzed the conformations of the phosphopantethiene moiety of coenzyme A, using molecular orbital calculations and conformational energy maps. They showed that this molecule has an intrinsic tendency to adopt conformations allowing hydrogen bonds between sulfhydryl, hydroxyl, carbonyl, amide, and phosphate groups. The conclusions agreed with the solution conformations deduced from NMR experiments of Lee & Sarma (1974, 1975) and Wilson et al. (1975). Our finding that the C-2 and C-8 protons of the adenine ring in S-trifluoroacetonyl-coenzyme A are shifted to lower field, relative to the values for the same protons in coenzyme A and

SCHEME I
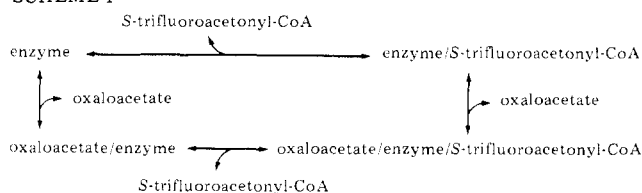
acetyl-coenzyme A, would also be consistent with a folded conformation (Mieyal et al., 1974, 1976).

α-Cyclodextrin, which is a ring of six $\alpha 1 \rightarrow 4$-linked D-glucose units, has a toroidal shape with a 5-Å diameter hydrophobic cavity (French, 1957; Bergeron & Rowan, 1976; Wood et al., 1977), and it is able to form inclusion complexes with a variety of nonpolar compounds. The large ^{19}F chemical shift it induces in S-trifluoroacetonylmercaptoethanol suggests the formation of such a complex, but it has a much smaller effect on S-trifluoroacetonyl-coenzyme A (Table II). Although the methylglucose lipopolysaccharide is postulated to have a helical, amylose-like conformation (Smith & Ballou, 1973; Rees, 1975) and might be expected to mimic α-cyclodextrin in its binding properties (Machida & Bloch, 1973; Bergeron et al., 1975), it induces only a small shift in the S-trifluoroacetonylmercaptoethanol or S-trifluoroacetonyl-coenzyme A resonances. The results imply that little binding occurs in these systems, or, if binding does occur, it produces little distortion of the electronic wave function in the vicinity of the ^{19}F nuclei. Our finding that acetyl-coenzyme A and n-hexanoyl-coenzyme A did not compete with S-trifluoroacetonyl-coenzyme A suggests that these short-chain analogues bind the lipopolysaccharide very weakly if at all. Moreover, they also fail to compete with S-trifluoroacetonylmercaptoethanol in its interaction with α-cyclodextrin, although benzoic acid, a compound known to form inclusion complexes with α-cyclodextrin, is a good competitor (Schlenk & Sand, 1961).

The binding of S-trifluoroacetonyl-coenzyme A and oxaloacetate to citrate synthetase can be represented as shown in Scheme I. Srere (1966) showed that oxaloacetate forms a stable binary complex with a dissociation constant of 0.6 μM. Our results give a dissociation constant for S-trifluoroacetonyl-coenzyme A in the ternary complex of about 1 mM by NMR measurements and a K_i of 0.16 mM by steady-state kinetic experiments. Considering the above scheme, these values should be equal. Several explanations can be offered for the discrepancy. First, the equation of Raftery et al. (1968) holds only when the ligand concentration is much greater than the site concentration of the macromolecule and is of the same order as the dissociation constant. Therefore, as mentioned previously, the dissociation constant extracted from the chemical shift data in Table III by the method of Raftery et al. (1968) could be in large error if a significant contribution to the observed ^{19}F chemical shift was due to nonspecific binding of S-trifluoroacetonyl-coenzyme A to the protein.

The formation of the binary S-trifluoroacetonyl-coenzyme A/enzyme complex results in a 17-Hz downfield shift and slight broadening of the ^{19}F signal. This suggests that the S-trifluoroacetonyl group is free to rotate, as it might be if located at the surface of the enzyme. This is consistent with studies of Weidman et al. (1973) who showed that a spin-labeled analogue of acetyl-coenzyme A, 3-carboxy-2,2,5,5-tetramethyl-1-pyrolidinyloxyl-coenzyme A thioester (R-coenzyme A), was only partially immobilized when bound to porcine heart citrate synthetase. Formation of the ternary S-trifluoroacetonyl-coenzyme A/enzyme/oxaloacetate complex by addition of oxaloacetate results in a further downfield shift and dramatic broadening of the ^{19}F signal. This suggests that the electronic

wave functions in the vicinity of the ^{19}F nuclei are further distorted and that the motion of the S-trifluoroacetonyl group becomes greatly restricted. One possibility is that the binding of oxaloacetate to the active site or secondary sites may induce a conformational change in the protein which brings the S-trifluoroacetonyl group into an electronic environment that deshields the ^{19}F nuclei and restricts its mobility. The results from two earlier studies support this hypothesis. Srere (1966) has shown that complex formation between oxaloacetate and citrate synthetase perturbs the ultraviolet and optical rotatory dispersion spectra of the enzyme, and Weidman et al. (1973) found that the rotational motion of the nitroxide moiety of the spin-labeled analogue of acetyl-coenzyme A (R-coenzyme A) was restricted when oxaloacetate was added to form the ternary R-coenzyme A/enzyme/oxaloacetate complex. Alternatively, from the equation given by Dwek (1973), which relates the observed resonance line width of a nucleus under rapid chemical exchange to its exchange life-time and the square of the chemical shift difference between the free and various bound states, one might expect from the data in Table III a $(47/17)^2$ increase in the line width even if the exchange lifetime and all motional parameters were constant.

Acknowledgments

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