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Enzyme-Catalyzed Synthesis of Fluoromalonyl-CoA

DAVID R. WALT*, JUNG-YIE KAO, AND TIANMEI OUYANG

Max Tishler Laboratory for Organic Chemistry, Department of Chemistry, Tufts University, Medford, Massachusetts 02155

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Acetyl-CoA carboxylase catalyzes the conversion of fluoroacetyl-CoA to fluoromalonyl-CoA. Fluoromalonyl-CoA was isolated and purified using reverse phase HPLC. The resulting purified compound was characterized with ^{19}F NMR.

INTRODUCTION

Substitution of fluorine for hydrogen in drugs often imparts enhanced pharmacological properties. In order to prepare these drug analogs, synthetic chemists have developed a variety of chemical methods for introducing fluorine into molecules (*1*). Some of these methods are harsh or not particularly selective. Fluorine can also be incorporated into organic compounds by providing enzymes with fluorinated substrate analogs (*2*). Fluorine's small size allows it to substitute readily at the enzyme's active site as long as unfavorable electrostatic interactions do not occur.

Fluoroacetic acid is known to be highly toxic. This toxicity is attributed to its *in vivo* conversion to fluorocitric acid which inhibits the aconitase reaction in the tricarboxylic acid cycle (*3*). We have shown previously that fluoroacetic acid is a substrate for the enzyme acetyl-CoA synthetase (ACS, EC 6.2.1.1) and is converted efficiently into fluoroacetyl-CoA (Eq. 1) (*4*)

$$FCH_2COOH + CoASH + ATP \xrightarrow{ACS} FCH_2COSCoA + AMP + PP_i \quad [1]$$

We now report that fluoroacetyl CoA is converted further by the enzyme acetyl-CoA carboxylase (ACC) to the previously unreported molecule fluoromalonyl-CoA—a compound with biochemical and synthetic significance.

METHODS

A typical reaction was carried out as follows: Fluoroacetic acid (15 μl, 260 μmol) and ATP (90 mg, 160 μl) [each dissolved separately in 0.5 ml of 0.5 M Tris buffer of pH 7.2] were added to the reaction mixture consisting of $MgCl_2$ (30 mg), CoA (25 mg, 32 μmol) and acetyl-CoA synthetase (2U) in Tris buffer, pH 7.2, at room temperature (final volume of the reaction solution was 2 ml). The reaction

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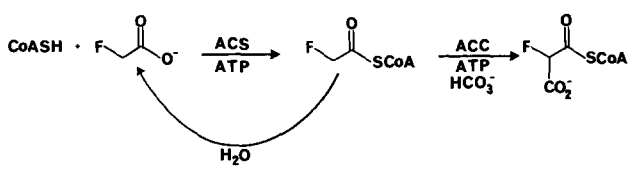
was incubated at room temperature and monitored using reverse phase HPLC (*6*). After 5 h, the CoA was nearly completely converted to fluoroacetyl-CoA. To the reaction was added $KHCO_3$ (2 M, 1 ml), sodium citrate (activator) (1.0 M, 50 μl), and the enzyme acetyl-coenzyme A carboxylase, which was extracted from rat liver (*7*), purified by affinity chromatography (*7, 8*), and assayed by HPLC. When the HPLC peak corresponding to fluoromalonyl-CoA was at a maximum, the solution was frozen and lyophilized. The lyophilized powder was dissolved in water and filtered and the filtrate was purified by successive injections on a preparative reverse phase C-8 HPLC column with 10–15% methanol–0.05 M phosphate, pH 5.5, as running buffer. The peak corresponding to fluoromalonyl-CoA was collected and lyophilized. ^1H NMR was taken after purification with HPLC and ^{19}F NMR was taken without separation.

RESULTS AND DISCUSSION

Biosynthetic pathways that employ acetate as a C_2 building block activate acetyl-CoA for condensation by increasing the acidity of the α-protons via enzyme-catalyzed carboxylation using acetyl-CoA carboxylase (ACC, EC 6.4.1.2) (*5*). The substrate analog fluoroacetyl-CoA is hydrolytically unstable (*4*). In order to provide ACC continuously with high concentrations of fluoroacetyl-CoA, a coupled enzyme system was employed (Scheme I). ACS was used to regenerate fluoroacetyl-CoA from its hydrolysis products, fluoroacetic acid and coenzyme A, using excess ATP to drive the reaction to completion.

The ^{19}F NMR spectra of the reaction products are shown in Fig. 1. (CCl_3F = 0 ppm). Spectrum A shows the ^{19}F spectrum of the starting fluoroacetyl-CoA as a triplet at 233.5 ppm. Spectrum B clearly shows the presence of a doublet at −184.3 ppm corresponding to fluoromalonyl-CoA. This chemical shift corresponds to that of fluoromalonic acid reported recently (*9*), and even more closely to α-monofluorinated β-diketones (*10*) (thioesters resemble ketones in their NMR spectral properties). The acidity of the α-proton was confirmed by exchange with D_2O. After 10 min the spectrum showed a peak growing at −184.9 ppm (spectrum C). After 8 h, further exchange had occurred as seen by the lack of observable H–F coupling constants (spectrum D). A high resolution ^{19}F NMR of spectrum D is shown in Fig. 2. The single peak at −184.9 ppm in spectrum D becomes a triplet in Fig. 2. The doublet at −184.3 ppm has J_{H-F} = 49.5 Hz while the triplet at −184.9 ppm has J_{D-F} = 9 Hz. This signal is attributed to the deuterated fluoromalonyl-CoA (*10*).



SCHEME 1

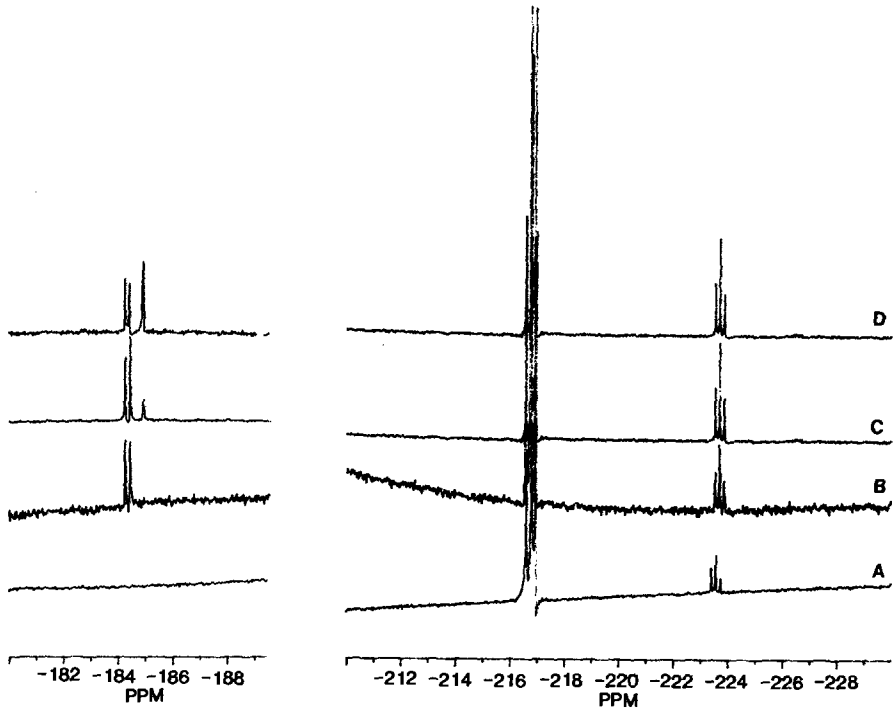


FIG. 1. ^{19}F NMR Spectra of (A) fluoroacetyl-CoA, (B) fluoromalonyl-CoA in H_2O, (C) fluoromalonyl-CoA in D_2O for 10 min, (D) fluoromalonyl-CoA in D_2O for 8 h.

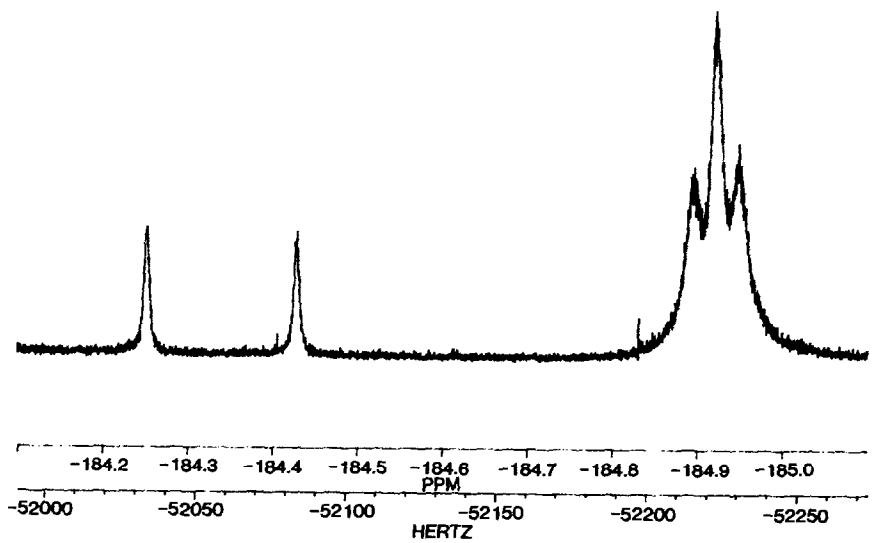


FIG. 2. High resolution ^{19}F NMR spectrum of fluoromalonyl-CoA in D_2O for 8 h.

The enzymatic synthesis of fluoromalonyl-CoA is significant biochemically because it suggests that the toxicity of fluoroacetic acid may result from a level different from only the citric acid cycle. From a synthetic standpoint, acetyl- and malonyl-CoA are the key starting materials in the fatty acid and polyketide biosynthetic pathways. If fluoroacetyl- and fluoromalonyl-CoA can substitute efficiently into these pathways, it should be possible to prepare fluorinated fatty acids and polyketide natural products using a chemoenzymatic route.

ACKNOWLEDGMENTS

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REFERENCES

1. Selected examples include: (a) MIDDLETON, W. J., (1975) *J. Org. Chem.* **40,** 574; (b) ROZEN, S., BRAND, M., ZAMIR, D., AND HEBEL, D., (1978) *J. Am. Chem. Soc.* **109,** 896–897; (c) ROZEN, S., AND BRAND, M., (1985) *Synthesis,* 665; (d) ROZEN, S., BRAND, M., AND KOL, M., (1989) *J. Am. Chem. Soc.* **111,** 8325–8326; (e) ROZEN, S., AND HEBEL, S., (1990) *J. Org. Chem.* **55,** 2621–2623; (f) LERMAN, O., AND ROZEN, S., (1983) *J. Org. Chem.* **48,** 724.
2. WALSH, C., (1983) *Adv. Enzymol.* **55,** 197–289.
3. PETERS, R., (1981) *Adv. Enzymol.* **20,** 647.
4. PATEL, S. S., AND WALT, D. R., (1987) *J. Biol. Chem.* **262,** 7132–7134.
5. WAKIL, S. J., STOOPS, J. K., AND JOSH, V. C., (1983) *Annu. Rev. Biochem.* **52,** 537–579.
6. (a) BAKER, F. C., AND SCHOOLEY, D. A., (1981) *in* Methods in Enzymology (Lowenstein, J. M., Ed.), Vol. 71, p. 41 Academic Press, San Diego; (b) INGBRETSEN, C., AND FARSTAD, M., (1980) *J. Chromatogr.* **202,** 439; (c) BAKER, F. C., AND SCHOOLEY, D. A., (1979) *Anal. Biochem.* **94,** 417.
7. THAMPY, K. G., AND WAKIL, S. J., (1985) *J. Biol. Chem.* **260,** 6318–6323.
8. BEATY, N. B., AND LANE, M. D., (1982) *J. Biol. Chem.* **257,** 920–924.
9. KITAZUME, T., SATO, T., KABAYASHI, T., AND LIN, J. T., (1986) *J. Org. Chem.* **51,** 1003–1006.
10. PURRINGTON, S. T., BUMGARDNER, C. L., LAZARIDIS, N. V., AND SINGH, P., (1987) *J. Org. Chem.* **52,** 4307–4310.