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T Tamura, M Wada, N Esaki and K Soda
J. Bacteriol. **1995, 177(9):2265.**

JOURNAL OF BACTERIOLOGY, May 1995, p. 2265–2269
0021-9193/95/$04.00+0

Vol. 177, No. 9

Synthesis of Fluoroacetate from Fluoride, Glycerol, and β-Hydroxypyruvate by *Streptomyces cattleya*

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Received 8 November 1994/Accepted 9 February 1995

Streptomyces cattleya **produces fluoroacetate and 4-fluorothreonine from inorganic fluoride added to the culture broth. We have shown by ^{19}F nuclear magnetic resonance (NMR) spectrometry that fluoroacetate is accumulated first in the culture broth and that accumulation of 4-fluorothreonine is next. To show precursors of the carbon skeleton of fluoroacetate, we carried out tracer experiments with various ^{14}C- and ^{13}C-labeled compounds. Radioactivity of [U-^{14}C]glucose, [U-^{14}C]glycerol, [U-^{14}C]serine, and [U-^{14}C]β-hydroxypyruvate was incorporated into fluoroacetate to an extent of 0.2 to 0.4%, whereas [3-^{14}C]pyruvate, [2,3-^{14}C]succinate, and [U-^{14}C]aspartate were less efficiently incorporated (0.04 to 0.08%). The addition of [2-^{13}C]glycerol to the mycelium suspension of *Streptomyces cattleya* caused exclusive enrichment of the carboxyl carbon of fluoroacetate with ^{13}C; about 40% of carboxyl carbon of fluoroacetate was labeled with ^{13}C. We studied the radioactivity incorporation of [3-^{14}C]-, [U-^{14}C]-, and [1-^{14}C]β-hydroxypyruvates to show that C-2 and C-3 of β-hydroxypyruvate are exclusively converted to the carbon skeleton of fluoroacetate. These results suggest that the carbon skeleton of fluoroacetate derives from C-1 and C-2 of glycerol through β-hydroxypyruvate, whose hydroxyl group is eventually replaced by fluoride.**

Fluoroacetate is one of the most toxic compounds produced by organisms (2, 3, 16). A few plants, *Dichapetalum cymosum* (6), *Palicourea marcgravii* (12), *Acacia georginae* (11, 14, 19), *Gastrolobium grandiflorum* (7), and *Oxylobium* species (1), are not only resistant to fluoroacetate but also synthesize it from fluoride at high concentrations. However, only speculative biosynthetic pathways of fluoroacetate in plants have been proposed, without evidence (8–10, 13, 18).

Sanada et al. (15) reported that *Streptomyces cattleya* NRRL 8057, a thienamycin producer, produces fluoroacetate and 4-fluorothreonine from the inorganic fluoride anion. However, it has not been shown whether fluoroacetate is first produced as a fluorinated metabolite or whether it is derived through 4-fluorothreonine.

In this study, we show by ^{19}F nuclear magnetic resonance (NMR) spectroscopy that fluoroacetate is first produced and metabolized to 4-fluorothreonine by *S. cattleya*. We have shown by experiments of incorporation of [2-^{13}C]glycerol and [3-^{14}C]-, [U-^{14}C]- and [1-^{14}C]β-hydroxypyruvates into fluoroacetate that glycerol and β-hydroxypyruvate are efficiently metabolized to form fluoroacetate, and we have proposed a biosynthetic pathway for fluoroacetate in *S. cattleya.*

MATERIALS AND METHODS

Materials. Radioisotopes were obtained from the following sources: L-[U-^{14}C]serine (5.22 GBq/mmol), [2-^{14}C]bromoacetic acid (2.07 GBq/mmol), D-[U-^{14}C]glucose (10.1 GBq/mmol), [2,3-^{14}C]succinic acid (4.07 GBq/mmol), and L-[U-^{14}C]aspartic acid (8.29 GBq/mmol) were from Amersham; DL-[3-^{14}C]serine (1.41 GBq/mmol) and DL-[1-^{14}C]serine (1.85 GBq/mmol) were from ICN; and [U-^{14}C]glycerol (0.37 GBq/mmol) and sodium [3-^{14}C]pyruvate (0.60 GBq/mmol) were from DuPont-NEN. [2-^{13}C]glycerol was purchased from MSD Isotopes (Montreal, Canada). [1,2-^{13}C]bromoacetate was from Cambridge Isotopes Laboratories. Sodium fluoroacetate was purchased from Wako Pure Chemicals (Osaka, Japan).

Microorganisms and cultivation. *S. cattleya* NRRL 8057 was obtained from the Northern Utilization Research and Development Division, U.S. Department of Agriculture. *S. cattleya* NTG29, which produced fluoroacetate at a concentration of 2.0 mM, was obtained by treatment of *S. cattleya* NRRL 8057 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The spore suspension (2×10^6 spores per ml of 20% glycerol) of the mutant strain was stored at −80°C until use. The cultivation was started by the addition of the spore suspension (5 ml) to the medium (5 ml), and the organism was grown at 37°C under aeration.

The following two kinds of growth media were used: medium PSM (1% potato extract, 2% sucrose, 2% malt extract [pH 6.5]) and medium K (2.5% soluble starch, 1.5% soybean flour, 0.2% yeast extract [pH 6.3]).

^{19}F NMR analysis of culture broth. Mycelia of *S. cattleya* NRRL 8057 grown on 5 ml of PSM medium supplemented with 10 mM KF for 5 days were again cultured in 500 ml of the same medium at 28°C under aeration. The culture broth (5 ml) was sampled at intervals, and mycelia were removed by centrifugation. The supernatant solution was concentrated under reduced pressure, dissolved in 0.5 ml of D_2O (99.8%; CEA, Gif-sur-Yvette, France), and analyzed by ^{19}F NMR. ^{19}F NMR spectra were obtained at 188 MHz with a Varian VXR-200 spectrometer with a Fourier transform accessory at ambient temperatures (20 to 24°C).

Synthesis of ^{14}C-labeled β-hydroxypyruvates. [3-^{14}C]-, [U-^{14}C]-, and [1-^{14}C]β-hydroxypyruvates were prepared from the corresponding ^{14}C-labeled serines by oxidative deamination with D-amino acid oxidase. The L-isomer was converted to the antipode in situ by means of the amino acid racemase with low substrate specificity (17). A solution (0.2 ml) containing [^{14}C]serine, 100 mM Tris-HCl buffer (pH 7.2), 3 U of D-amino acid oxidase, 5 U of amino acid racemase with low substrate specificity, and 1.3 U of catalase was incubated at 30°C for 70 min and then applied to a Dowex 50X8 (H^+) column (volume, 1 ml). The column was washed with 0.1 ml of 100 mM Tris-HCl buffer (pH 7.2) containing 10 mg of unlabeled β-hydroxypyruvate. The eluate was subjected to preparative high-performance liquid chromatography (HPLC) under the conditions described below. [U-^{14}C]-, [1-^{14}C]-, and [3-^{14}C]β-hydroxypyruvates were obtained with yields of 78, 76, and 98%, respectively. The specific radioactivity of the product was adjusted to 330 MBq/mmol by the addition of unlabeled β-hydroxypyruvate.

Synthesis of [2-^{14}C]glycolate and [1,2-^{13}C]glycolate. A solution containing [2-^{14}C]bromoacetic acid (9.25 MBq), 5.4 mmol of calcium carbonate, and 50 mmol of sodium hydroxide in 5 ml of water was refluxed at 100°C for 4 h. [2-^{14}C]glycolate was purified by preparative HPLC as described below: the yield was 23%, on the basis of the recovery of radioactivity (2.16 MBq). The specific radioactivity was adjusted to 330 MBq/mmol by the addition of unlabeled glycolate.

A solution containing [1,2-^{13}C]bromoacetic acid (1 g; 7.2 mmol) and potassium hydroxide (580 mg; 14.5 mmol) in 15 ml of water was autoclaved in a sealed vial at 120°C for 15 min. The solution was concentrated to 3 ml by evaporation, adjusted to pH 2 with 1 N HCl, and loaded on an Extrelute column (Merck). [1,2-^{13}C]glycolate was eluted with 100 ml of ethyl ether and obtained by evaporation of the solvent as a white powder (385 mg; 5 mmol), with a yield of 69%. ^1H NMR (D_2O), δ 4.25 (dd, J = 4.4 Hz, 14.4 Hz); ^{13}C NMR (D_2O), δ 59.2 (d, J = 59 Hz), δ 176.3 (d, J = 59 Hz).

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Addition of ^{14}C-labeled compounds to growing and resting mycelia of *S. cattleya* NTG29. (i) Experiment 1. Tracer experiments with growing mycelia were carried out by the addition of a ^{14}C-labeled compound to 50 ml of the culture broth grown on medium K supplemented with 10 mM KF for 5 days. After incubation at 37°C for 12 h, fluoroacetate was isolated as described below.

(ii) Experiment 2. Tracer experiments with resting mycelia were carried out with mycelia grown on 50 ml of medium K supplemented with 10 mM KF for 5 days under aeration. The harvested mycelia were washed three times with 50 mM MES (morpholinoethanesulfonic acid; pH 6.5) and suspended in 25 ml of the same buffer. The mycelial suspension was supplemented with 10 mM KF and shaken at 37°C for 4 h. ^{14}C-labeled β-hydroxypyruvate was added to the suspension and incubated at 37°C for 3 h under aeration. Fluoroacetate was isolated as described below.

(iii) Experiment 3. Permeabilized mycelia of *S. cattleya* NTG29 were used to examine the incorporation of [2-^{14}C]glycolate. The mycelia were grown in medium K supplemented with 10 mM KF. The mycelial suspension prepared as described above was cooled in an ice bath and stirred vigorously with 0.2% (wt/vol) benzyl alcohol for 30 s. The mycelia were collected by centrifugation and suspended in 25 ml of 50 mM MES buffer (pH 6.5) supplemented with 10 mM KF. After the suspension was incubated at 37°C for 4 h under aeration, [2-^{14}C] glycolate was added to the suspension. Fluoroacetate was isolated after incubation at 37°C for 12 h under aeration.

Isolation of fluoroacetate. The mycelia grown were removed by centrifugation and washed with the same volume of water. The supernatant solution was concentrated to 5 ml under reduced pressure and mixed with 5 ml of ethanol. Precipitate was removed by centrifugation, and ethanol was removed by evaporation. NaCl (0.1 g) was added to the remaining solution, which was washed three times with 10 ml of ether. The aqueous layer was then acidified by the addition of 1 ml of 20% H_2SO_4 and extracted five times with 20 ml of ether. The ether layer was combined, mixed with 1 ml of 0.1 M $NaHCO_3$, and evaporated under reduced pressure. The residue (about 1 ml) was acidified with 1 ml of 65% $HClO_4$ and subjected to preparative HPLC under the following conditions. An ULTRON PS-80H column (0.8 by 30 cm-; Shinwa Kako, Kyoto, Japan) was kept in a column oven at 60°C. The mobile phase was distilled water whose pH was adjusted at 2.1 with H_2SO_4, and the flow rate was 1.0 ml/min. The eluate was monitored at 210 nm. The radioactivity of fluoroacetate was measured in 10 ml of Clearsol I (Nacalai Tesque, Kyoto, Japan) with a Packard A300CD liquid scintillation counter.

Addition of [2-^{13}C]glycerol to a mycelium suspension of *S. cattleya* NTG29. *S. cattleya* NTG-29 was grown in 250 ml of medium K supplemented with 10 mM KF at 37°C for 5 days under aeration. The harvested mycelia were washed three times with 500 ml of 50 mM MES buffer (pH 6.5) and suspended in 250 ml of the same buffer containing 10 mM KF and 10 mM [2-^{13}C]glycerol. The suspension was incubated at 37°C for 72 h under aeration. After the mycelia were removed by centrifugation, the supernatant solution was concentrated to 5 ml, acidified with sulfuric acid, and extracted five times with ether (20 ml). The ether extract was analyzed by proton-decoupled ^{13}C NMR. Fluoroacetate was isolated by preparative HPLC as described above and analyzed by gas chromatography-mass spectrometry (GC-MS) and ^{1}H and ^{19}F NMR.

GC-MS analysis. Eluate containing fluoroacetate was evaporated to dryness, and the residue was dissolved in 0.1 ml of 20% H_2SO_4 and extracted twice with 0.5 ml of ether. The extract was mixed with 0.1 ml of methanol, and ether was removed by evaporation. The remaining solution was filled up to 0.5 ml with ether containing about 2% (wt/vol) diazomethane. A 2-ml aliquot of the solution was analyzed with a Varian 3400 gas chromatograph directly connected with an ion trap detector ITD-800 (Finigan MAT Instrument Inc.). A capillary column (0.25 mm by 50 m; ULTRON HR-20) was kept at 50°C for 1 min, and then the temperature was raised to 71°C at a rate of 3.5°C/min; fluoroacetate was eluted at 6.3 min.

RESULTS

Biosynthesis of fluoroacetate and 4-fluorothreonine by *S. cattleya* NRRL 8057. We analyzed the culture broth of *S. cattleya* NRRL 8057 by ^{19}F NMR (Fig. 1). A triplet signal appeared at −217 ppm and was assigned to be the ^{19}F NMR signal of fluoroacetate by comparison with the standard sodium fluoroacetate. 4-Fluorothreonine was reported to show a double triplet signal at −231.6 ppm (15), and the corresponding signal was observed 3 days after the appearance of the fluoroacetate signal. We observed no other ^{19}F NMR signals in the culture broth. These results strongly suggest that fluoroacetate is first produced and then metabolized to 4-fluorothreonine by *S. cattleya* NRRL 8057.

Incorporation of radioactivity of ^{14}C-labeled compounds into fluoroacetate. We carried out tracer experiments in order to show the biosynthetic pathway of fluoroacetate in *S. cattleya*. Several compounds labeled with ^{14}C and participating in the glycolytic pathway, the tricarboxylic acid cycle, and related pathways were added to the culture broth. Fluoroacetate was isolated from the culture, and radioactivity incorporated was measured (Table 1, experiment 1). [U-^{14}C]glycerol was the compound whose radioactivity was incorporated most efficiently and was followed in efficiency by [U-^{14}C]glucose, [U-^{14}C]β-hydroxypyruvate, and L-[U-^{14}C]serine. Radioactivity of ^{14}C-labeled pyruvate, L-aspartate, and succinate was also incorporated but much less efficiently than for the above-listed compounds.

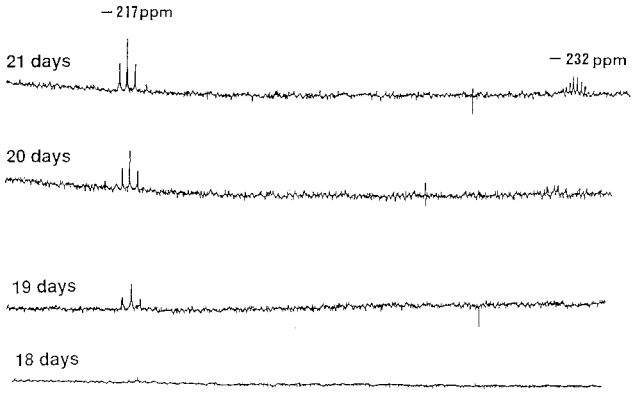

FIG. 1. ^{19}F NMR analysis of the culture broth of *S. cattleya* NRRL 8057. The mycelia were grown in medium PSM supplemented with 10 mM KF at 28°C as described in Materials and Methods. The culture broth was taken at intervals, and ^{19}F NMR of the supernatant solution was measured. Chemical shifts upfield from the signal of trichlorofluoromethane are expressed.

We used resting mycelia of *S. cattleya* to show the incorporation of the radioactivity of ^{14}C-labeled β-hydroxypyruvate into fluoroacetate (Table 1, experiment 2). The incorporation rate of the radioactivity of [U-^{14}C]β-hydroxypyruvate was about 20% lower than that of [3-^{14}C]β-hydroxypyruvate. In contrast, only a little of the radioactivity of [1-^{14}C]β-hydroxypyruvate was incorporated into fluoroacetate. This shows that only C-2 and C-3 of β-hydroxypyruvate are incorporated into fluoroacetate.

Incorporation of [2-^{13}C]glycerol into fluoroacetate with mycelia of *S. cattleya* NTG29. When [2-^{13}C]glycerol was incubated with *S. cattleya* NTG29 mycelia, ^{13}C was incorporated into fluoroacetate. Methyl fluoroacetate prepared from standard sodium fluoroacetate shows peaks of *m/z* 93 (M + 1) and 61 (M − OMe) on GC-MS analysis (Fig. 2A). However, the ^{13}C-labeled fluoroacetate produced by the mycelia gave peaks at *m/z* 94 and 62 in addition to those at *m/e* 93 and 61 (Fig. 2B). This shows that fluoroacetate enriched with one ^{13}C atom was produced by the mycelia.

Standard sodium fluoroacetate gave two doublet signals on the proton-decoupled ^{13}C NMR spectrum: δ 80 ppm ($J_{C\text{-}F}$ = 177 Hz) for FCH_2 and δ 178 ppm ($J_{C\text{-}F}$ = 18.7 Hz) for -COONa (Fig. 3A). The ^{13}C-labeled-fluoroacetate produced showed only one major ^{13}C signal at 178 ppm (Fig. 3B). This indicates that ^{13}C was incorporated exclusively into the carboxyl carbon of fluoroacetate.

No ^{19}F NMR signals showing a heteronuclear coupling with ^{13}C could be observed by ^{19}F NMR because of a low natural abundance of ^{13}C. However, the ^{19}F NMR spectrum of the ^{13}C-labeled fluoroacetate showed a coupling of 18.7 Hz (Fig. 4A). This is consistent with the coupling constant observed by proton-decoupled ^{13}C NMR as described above. This also indicates the exclusive incorporation of ^{13}C into the carboxyl carbon of fluoroacetate.

TABLE 1. Incorporation of ^{14}C-labeled substrates into monofluoroacetate and other compounds by *S. cattleya* NTG29[a]

Expt and precursor	Radioactivity of precursors added (Bq) (*A*)	Specific radioactivity of precursors (Bq/nmol) (*B*)	Radioactivity of fluoroacetate isolated (Bq) (*C*)	Specific radioactivity of fluoroacetate isolated (Bq/nmol) (*D*)	Ratio (10^3)	
					C/*A*	*D*/*B*
Expt 1[b]						
D-[U-^{14}C]glucose	56,000	10,000	220	4.5	40	0.45
[3-^{14}C]pyruvate	63,000	600	38	0.76	0.6	1.3
L-[U-^{14}C]aspartate	48,000	8,300	38	0.79	0.8	0.095
[2,3-^{14}C]succinate	78,000	4,100	31	0.66	0.4	0.16
[U-^{14}C]glycerol	81,000	370	340	6.8	4.2	18
L-[U-^{14}C]serine	49,000	5,200	110	2.2	2.2	0.42
[U-^{14}C]β-hydroxypyruvate	39,000	330	90	1.8	2.3	5.5
Expt 2[c]						
[3-^{14}C]β-hydroxypyruvate	68,000	330	1,200	ND[d]	17	ND
[U-^{14}C]β-hydroxypyruvate	56,000	330	730	ND	13	ND
[1-^{14}C]β-hydroxypyruvate	74,000	330	52	ND	0.7	ND
Expt 3[e]						
[2-^{14}C]glycolate	39,000	330	27	ND	0.7	ND

[a] Mycelia of *S. cattleya* NTG29 were grown in medium K supplemented with 10 mM KF for 5 days as described in Materials and Methods.
[b] Labeled precursors were added to the culture broth 5 days after inoculation. The culture was incubated with shaking at 37°C for 12 h and then sonicated with a Biomic ultrasonic disintegrator for 10 min. Fluoroacetate was isolated from the supernatant solution by preparative HPLC as described in Materials and Methods.
[c] The resting mycelia were used. Incubation time was 3 h.
[d] ND, not determined.
[e] The permeabilized mycelia were used.

The ^1H NMR spectrum of the fluoroacetate sample isolated from the mycelial culture incubated with [2-^{13}C]glycerol showed a couple of peaks on either side of both major peaks at 4.72 and 4.47 ppm (Fig. 4B). The side peaks correspond to the signals of β-methylene protons of ^{13}C-labeled fluoroacetate, whereas the major peaks are those of nonlabeled fluoroacetate. We determined the relative concentrations of ^{13}C-labeled and nonlabeled fluoroacetates formed to be about 0.4 on the basis of the relative peak intensities of these signals. This indicates that about 40% of the fluoroacetate molecules formed were labeled with ^{13}C exclusively at the carboxyl group. Fifty micromoles of fluoroacetate was produced in the system containing 2.5 mmol of [2-^{13}C]glycerol. Accordingly, about 0.8% of [2-^{13}C]glycerol added was metabolized to form [1-^{13}C] fluoroacetate. Thus, we have concluded that the precursor of fluoroacetate is derived mainly from glycerol. The exclusive incorporation of both C-2 and C-3 of β-hydroxypyruvate into fluoroacetate suggests that fluoroacetate is synthesized through β-hydroxypyruvate or its derivative by *S. cattleya* mycelia, which metabolize glycerol to β-hydroxypyruvate as well.

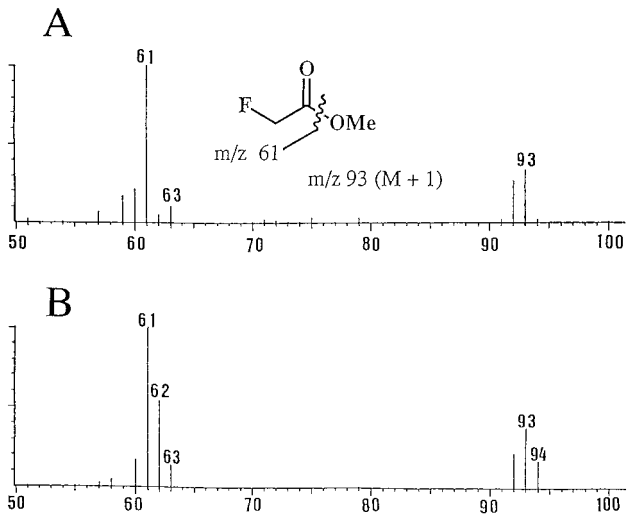

FIG. 2. GC-MS analysis of the authentic methyl fluoroacetate (A) and the methyl ester of the fluoroacetate sample isolated from the mycelial culture incubated with [2-^{13}C]glycerol (B).

Incorporation of ^{14}C and ^{13}C of [2-^{14}C] and [1,2-^{13}C]glycolate into fluoroacetate. The above results suggest that β-hydroxypyruvate (or its derivatives) undergoes conversion to fluoroacetate; a substitution of fluoride for the hydroxyl group and a decarboxylation occur. There are two possible pathways for the conversion, according to the order of these reactions. β-Hydroxypyruvate may be first decarboxylated to form glycolate or its derivative and then converted to fluoroacetate. Alternatively, β-hydroxypyruvate may be first converted to β-fluoropyruvate and then decarboxylated to form fluoroacetate. We obtained no evidence to show the formation of β-fluoropyruvate in the culture broth of *S. cattleya* by ^{19}F NMR as described above. Accordingly, we examined whether glycolate is the precursor of fluoroacetate by tracer experiments. In order to exclude the possibility that glycolate is not permeabilized through the membranes of *S. cattleya* mycelia, we used permeabilized mycelia of *S. cattleya* NTG-29. The permeabilization treatment affected the fluoroacetate production only slightly: the treated mycelia produced fluoroacetate only 30% less than the native mycelia (data not shown). However, radioactivity of [2-^{14}C]glycolate was incorporated into fluoroacetate only a little (Table 1, experiment 3). Likewise, fluoroacetate was not enriched with ^{13}C in the system containing [1,2-^{13}C] glycolate. Thus, we have concluded that glycolate is not the precursor of fluoroacetate.

DISCUSSION

We have shown the main pathway of fluoroacetate biosynthesis in *S. cattleya* to be through β-hydroxypyruvate or its close derivative. This is the first report of fluoroacetate biosynthesis shown on the basis of tracer experiments with labeled com-

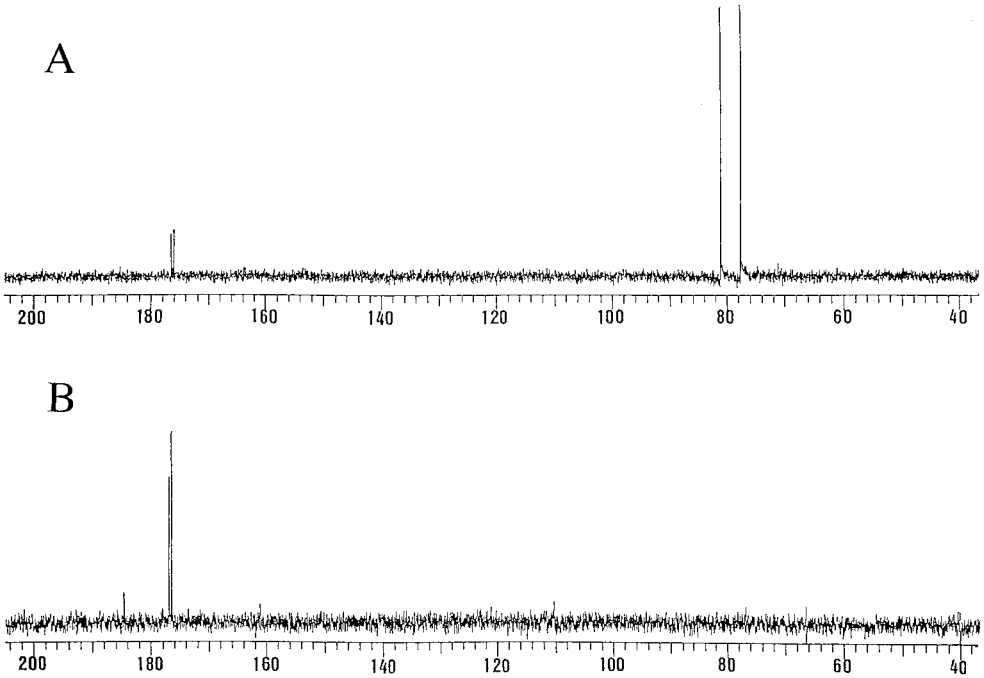

FIG. 3. Proton-decoupled ^{13}C NMR spectra of the authentic sodium fluoroacetate (A) and the fluoroacetate sample isolated from the mycelial culture incubated with [2-^{13}C]glycerol (B).

pounds. Peters et al. showed that fluoroacetate is produced by the incubation of pyruvate, potassium fluoride, and ATP with a homogenate of *A. georginae* seedlings (13). However, the radioactivity of ^{14}C-labeled pyruvate was not incorporated efficiently into fluoroacetate in *S. cattleya*. *A. georginae* may differ from *S. cattleya* in the biosynthetic pathway of fluoroacetate, but the plant pathway remains to be examined by tracer experiments.

The halide ions Cl^-, Br^-, and I^- are usually incorporated into organic compounds such as alkenes by the action of haloperoxidases in living systems (4, 5). Vickery et al. speculated that fluoroacetate may be formed from malonate, fluoride, and hydrogen peroxide by a haloperoxidase (18). However, the redox potential for reduction of hydrogen peroxide (E_0 = +1.77 V) is insufficient to oxidize the fluoride anion (E_0 = −3.06 V): the C-F bond cannot be formed by haloperoxidase reactions. Therefore, fluoroacetate is probably synthesized by the replacement of a leaving group of a substrate by a fluoride anion by the action of an enzyme, possibly a housekeeping enzyme occurring in *S. cattleya* mycelia and catalyzing by chance the fluoride incorporation reaction.

Meyer et al. (9, 10) observed a conversion of β-fluoropyruvate to fluoroacetate by incubation with the extract of *D. cymosum*, a fluoroacetate-producing plant, and proposed that β-fluoropyruvate is the direct precursor of fluoroacetate. However, Baron et al. (1) failed to detect β-fluoropyruvate in the extracts of seeds and foliage of 20 species of fluoroacetate-producing plants by ^{19}F NMR. We also could not show accumulation of β-fluoropyruvate in the culture broth of *S. cattleya*. The minimum concentration of fluoroacetate that could be clearly detected by ^{19} F NMR was about 20 mg/ml under the conditions we used. Even if β-fluoropyruvate was produced, its concentration was probably lower than 20 mg/ml in the culture.

Mead and Segal (8) suggested that fluoroacetate is produced from serine or its derivatives such as *O*-acetylserine and *O*-phosphoserine by replacement of the β-substituent group by

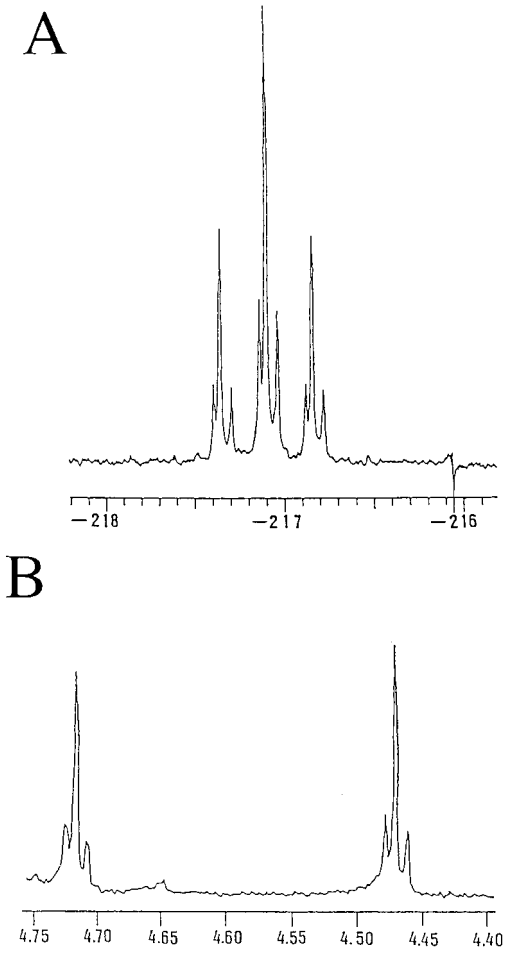

FIG. 4. ^{19}F NMR (A) and ^{1}H NMR (B) spectra of the fluoroacetate sample isolated from the mycelial culture incubated with [2-^{13}C]glycerol.

fluoride catalyzed by a pyridoxal 5′-phosphate enzyme. The β-fluoroalanine supposed to be formed is possibly converted to β-fluoropyruvate, which may be decarboxylated to form fluoroacetate through an enzymatic transamination or oxidation. However, our present results showing the participation of β-hydroxypyruvate do not make accommodations for this pathway, and no evidence has been obtained for the formation of β-fluoroalanine. Even if β-fluoropyruvate was formed, its concentration should be lower than the detection limit by ^{19}F NMR. Whatever may be the reaction of fluoroacetate biosynthesis and whatever may be the physiological function of the enzyme participating in the reaction, the enzyme catalyzing the incorporation of fluoride into the precursor of fluoroacetate is unique in that it acts on the fluoride anion, which is usually inert in water because of a strong solvation with water.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid for Scientific Research (02662116) from the Ministry of Education, Science and Culture of Japan to N.E. and by a grant from the Japan Foundation for Applied Enzymology to K.S.

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