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Evidence for *cis-trans* Isomerization of a Double Bond in the Fatty Acids of the Psychrophilic Bacterium *Vibrio* sp. Strain ABE-1

NAOKI MORITA,[1] AKIRA SHIBAHARA,[2] KOHEI YAMAMOTO,[2] KAORI SHINKAI,[2] GORO KAJIMOTO,[2] AND HIDETOSHI OKUYAMA[1]*

Department of Biology, Faculty of Science, Hokkaido University, Kita-ku, Sapporo 060,[1] and Department of Nutrition, Kobe-Gakuin University, Nishi-ku, Kobe 651-21,[2] Japan

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***Vibrio* sp. strain ABE-1 was grown in a medium that contained as its stable isotope tracer either [2,2-$^2H_2$]*cis*-9-hexadecenoic or [2,2-$^2H_2$]*trans*-9-hexadecenoic acid. Gas chromatographic-mass spectrometric analysis of the *cis*-9-hexadecenoic and *trans*-9-hexadecenoic acid fractions from the cells revealed the formation of an intracellularly isomerized 2,2-$^2H_2$-fatty acid which differed from the tracer only in the geometrical configuration of the double bond. This observation shows that *cis-trans* isomerization without a shift in double-bond position between these two geometric hexadecenoic acid isomers can occur in the cells.**

Okuyama et al. (8) found that the lipids of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1, contain high levels of a unique *trans*-9-hexadecenoic acid [16:1(9t)]. The biosynthesis of this *trans*-acid is dependent on growth temperature (8). When *Vibrio* sp. strain ABE-1 cells grown initially at 5°C are transferred to medium at 20°C and then grown at this temperature in the presence of cerulenin (9), the level of 16:1(9t) increases concomitantly with a decrease in the level of *cis*-9-hexadecenoic acid [16:1(9c)] (7). Therefore, Okuyama et al. (7) suggested that 16:1(9t) is synthesized by *cis-trans* isomerization of 16:1(9c) in *Vibrio* sp. strain ABE-1. However, direct evidence of *cis-trans* isomerization between 16:1(9c) and 16:1(9t) has not yet been obtained.

In a recent study, Shibahara et al. (12) presented novel pathways for the biosynthesis of *cis*-9-octadecenoic acid [18:1(9c)] and *cis*-11-octadecenoic acid [18:1(11c)] in higher plants. These monoenoic acids are formed by enzymatic reactions that involve a shift in the location of the double bond [i.e., 18:1(9c) from 18:1(11c) and 18:1(11c) from 18:1(9c)]. Such reactions were monitored by tracing the fate of [2,2-$^2H_2$]18:1(9c) or [2,2-$^2H_2$]18:1(11c) by gas chromatographic-mass spectrometric (GC-MS) analysis. The GC-MS method in which 2,2-$^2H_2$-fatty acids are used as tracers (11, 12) is a useful technique for studying the metabolism of positional and also geometrical isomers of fatty acids.

In this study, we examined in vivo conversion between 16:1(9c) and 16:1(9t) in *Vibrio* sp. strain ABE-1 by using 2,2-$^2H_2$-fatty acids as stable isotope tracers in order to determine whether the conversion is direct.

Vibrio sp. strain ABE-1 (13) was grown with shaking at 15°C in a Tris-buffered medium (7). Five-milliliter portions of the suspension of cells at the middle of the logarithmic phase of growth were transferred to 100 ml of a Tris-buffered medium that contained 0.5% (wt/vol) Triton X-100 and 0.2% (wt/vol) [2,2-$^2H_2$]16:1(9c) or 0.2% [2,2-$^2H_2$]16:1(9t) and then were incubated with shaking for 12 h at 15°C for labeling of the cells with [2,2-$^2H_2$]16:1(9c) and for 12 h at 10°C for labeling of the cells with [2,2-$^2H_2$]16:1(9t). The addition of 0.5% (wt/vol) Triton X-100 to the medium had no effect on the growth of this bacterium. After being incubated, cells in 10-ml portions of the cultures were harvested by centrifugation at 5,000 × g for 10 min and were then washed with 0.5 M NaCl.

The total lipids were extracted from the medium-free cells by using the method of Bligh and Dyer (1) and then heated with 10% (vol/vol) acetyl chloride in methanol at 90°C for 3 h in the presence of a known amount of heptadecanoic acid as an internal standard. The resultant methyl esters were extracted with hexane. A small aliquot of the esters was analyzed by capillary gas-liquid chromatography (6) for estimation of the qualitative and quantitative compositions of the fatty acids. The remaining esters were then separated into saturated, *trans*-monoenoic, and *cis*-monoenoic fractions by silver nitrate thin-layer chromatography on silica gel plates (E. Merck, Darmstadt, Germany) with the solvent system chloroform-ethanol (99:1 [vol/vol]) (8). After the plates were sprayed with 0.01% (wt/vol) primurine in a mixture of acetone-water (4:1 [vol/vol]), the bands of methyl esters were detected under UV light (14). The bands of *trans*- and *cis*-monoenoates were scraped off and extracted with a mixture of hexane–methanol–0.5 M NaCl (2:1:1 [vol/vol/vol]). The recovered *trans*- and *cis*-monoenoates were subjected separately to the I_2-catalyzed reaction for the formation of adducts with dimethyl disulfide according to the procedure of Shibahara et al. (10) with minor modifications (15). The resultant dimethyl disulfide adducts were analyzed by the GC-MS method under conditions described previously (11).

Authentic 16:1(9c) and 16:1(9t) were obtained from Nu-Chek Prep, Inc. (Elysian, Minn.). Their purities were checked by silver nitrate thin-layer chromatography, capillary gas-liquid chromatography, and GC-MS analysis and were found suitable for the present study. The 2,2-$^2H_2$-fatty acids were prepared by using published methods (4, 5). Heptadecanoic acid was purchased from Sigma Chemical Co. (St. Louis, Mo.). All other reagents were of analytical grade.

Figure 1B and C show the mass spectra of 16:1(9t) and 16:1(9c) isolated from cells labeled with [2,2-$^2H_2$]16:1(9c) and [2,2-$^2H_2$]16:1(9t), respectively, for 3 h. As shown in Fig. 1B, the molecular ion at *m/z* 364 (M^+) and the fragment ions

* Corresponding author.

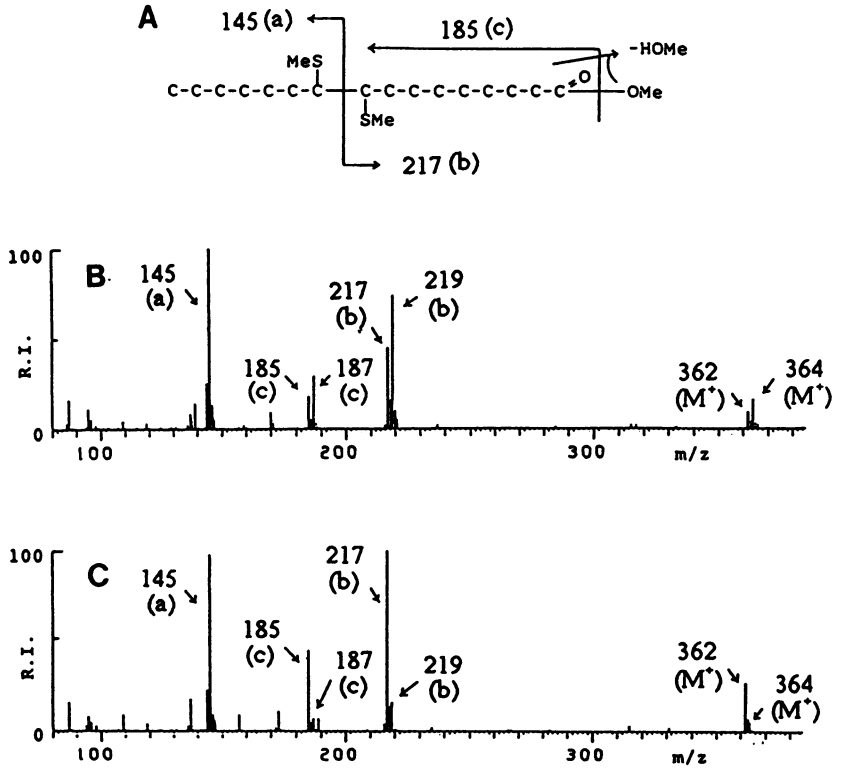

FIG. 1. Mass spectra of dimethyl disulfide adducts of total 16:1(9t) methyl esters prepared from cells labeled with [2,2-$^2H_2$]16:1(9c) at 15°C (B) and total 16:1(9c) methyl esters prepared from cells labeled with [2,2-$^2H_2$]16:1(9t) at 10°C (C) for 3 h. The common fragmentation pattern of the dimethyl disulfide adduct of methyl esters of nonlabeled 16:1(9c) and 16:1(9t) is also shown (A). a, b, and c, fragment ions. Relative intensity (RI) is expressed as a percent.

(c and b) (Fig. 1A) at *m/z* 187 and 219 were evident and were stronger than the corresponding ions of nonlabeled 16:1(9t), that is, the molecular ion at *m/z* 362 and the fragment ions (c and b) at *m/z* 185 and 217. These results demonstrate that [2,2-$^2H_2$]16:1(9c) is taken up by the cells and subsequently converted to [2,2-$^2H_2$]16:1(9t). By contrast, molecular and fragment ions 2 mass units larger were not so abundant in the 16:1(9c) fraction from cells labeled with [2,2-$^2H_2$]16:1(9t) (Fig. 1C). Thus, in cells grown for 12 h, the labeled geometrical isomer formed from [2,2-$^2H_2$]16:1(9c), 16:1(9t), accounted for 77% of the total 16:1(9t) (Table 1), and the labeled geometrical isomer, formed from [2,2-$^2H_2$]16:1(9t), 16:1(9c), accounted for 5% of the total 16:1(9c) (Table 2). Control experiments with nonlabeled 16:1(9c) and 16:1(9t) did not reveal the presence of any label from $^2H_2$-fatty acids in the 16:1(9t) and 16:1(9c) fractions of cells, respectively (Tables 1 and 2). The GC-MS profiles of completely hydrogenated esters of intracellularly isomerized 16:1(9t) and 16:1(9c) that originated from 2,2-$^2H_2$-substrates revealed a specific fragment ion at *m/z* 76 (12), not at *m/z* 74, which was because of McLafferty rearrangement (4) (data not shown). This observation indicates that the 16:1(9t) formed from [2,2-$^2H_2$]16:1(9c) and the 16:1(9c) formed from [2,2-$^2H_2$]16:1(9t) have two deuterium atoms at the second carbon atom from the carboxy end in the molecules. A combination of β-oxidation and de novo resynthesis of fatty acid as an explanation for the production of dideuterated fatty acid with a specific labeling pattern (see above) can be eliminated, as discussed previously (11). Our results show that *cis-trans* isomerization between 16:1(9c) and 16:1(9t) occurs in vivo in *Vibrio* sp. strain ABE-1.

It is known that 16:1(9c) is generated in this bacterium by

TABLE 1. Levels of $^2H_2$-labeled 16:1(9t) prepared from total lipids of *Vibrio* sp. strain ABE-1 cells labeled with [2,2-$^2H_2$]16:1(9c)

Substrate[a]	Total 16:1(9t) (nmol/10 ml of culture)[b]	Labeled 16:1(9t) (nmol/10 ml of culture)[c]
[2,2-$^2H_2$]16:1(9c)	130	100 (77)
16:1(9c)[d]	168	0 (0)

[a] The labeling time for both was 12 h.
[b] Errors in detecting the amounts of fatty acid were less than 15%.
[c] Numbers in parentheses are percentages of the total 16:1(9t).
[d] Unlabeled addition served as the negative control for the deuterated signals.

TABLE 2. Levels of $^2H_2$-labeled 16:1(9c) prepared from total lipids of *Vibrio* sp. strain ABE-1 cells labeled with [2,2-$^2H_2$]16:1(9t)

Substrate[a]	Total 16:1(9c) (nmol/10 ml of culture)[b]	Labeled 16:1(9c) (nmol/10 ml of culture)[c]
[2,2-$^2H_2$]16:1(9t)	350	18 (5)
16:1(9t)[d]	401	0 (0)

[a] The labeling time for both was 12 h.
[b] Errors in detecting the amounts of fatty acid were less than 15%.
[c] Numbers in parentheses are percentages of the total 16:1(9c).
[d] Unlabeled addition served as the negative control for the deuterated signals.

both the aerobic pathway, i.e., the desaturation of palmitic acid (16:0), and the anaerobic pathway, i.e., de novo synthesis by type II fatty acid synthetase (2, 3). Furthermore, the equilibrium of *cis-trans* isomerization between 16:1(9c) and 16:1(9t) lies in the direction of the conversion of the *cis* isomer to the *trans* isomer (Tables 1 and 2). Therefore, *trans*-to-*cis* isomerization seems not to be an essential route for the generation of 16:1(9c). The possibility that 16:1(9t) can be synthesized de novo by *trans* desaturation of 16:0 can be eliminated, because when *Vibrio* sp. strain ABE-1 was grown in the presence of cerulenin (9) under aerobic conditions, the synthesis of 16:1(9t) was accompanied by a decrease in the level of 16:1(9c) but not in that of 16:0 (7). Thus, *cis*-to-*trans* isomerization of 16:1(9c) must be the sole biosynthetic pathway for generation of 16:1(9t) in this bacterium.

Okuyama et al. (7) suggested that isomerization between 16:1(9c) and 16:1(9t) is catalyzed by a *cis-trans* isomerase(s) in *Vibrio* sp. strain ABE-1. In this study, 2,2-$^2H_2$-fatty acids were added to the culture medium in the free-acid form. It is unknown, however, whether this hypothetical isomerase(s) responsible for the isomerization utilizes the substrate in the free-acid form or in an acylated form bound to phospholipids. Fatty acid thioesters of coenzyme A and/or acyl carrier protein may serve as the actual substrate for isomerization. It has been suggested previously that 16:1(9t) is formed by isomerization of 16:1(9c) at the *sn*-2 position of phosphatidylethanolamine because 16:1(9t) is specifically located at the *sn*-2 position (7). The hypothetical enzyme is unique in catalyzing the configurational isomerization of a double bond without a positional shift of the double bond (7). In order to obtain further biochemical information on *cis-trans* isomerization, it will be necessary to establish an in vitro assay system in which the isomerase(s) would be active.

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