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Fatty Acid Synthesis of an Eicosapentaenoic Acid-Producing Bacterium: *De Novo* Synthesis, Chain Elongation, and Desaturation Systems

Kazuo Watanabe,*[,1] Kazunaga Yazawa,* Kiyosi Kondo,* and Akihiko Kawaguchi†

Sagami Chemical Research Center, Nishi-Ohnuma 4-4-1, Sagamihara, Kanagawa 229; and †Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153

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The fatty acid synthesis systems of a *Shewanella* sp., strain SCRC-2738, that produces a large amount of eicosapentaenoic acid were investigated. Two kinds of fatty acid synthesis system, *de novo* synthesis and chain elongation ones, were detected in the cytosol. The *de novo* synthesis system required an acyl carrier protein, and produced palmitoyl- and palmitoleoyl-acyl carrier proteins as final products. The chain elongation system also required an acyl carrier protein, and produced an acyl-acyl carrier protein as a product, using palmitoyl-, palmitoleoyl-, stearoyl-, and oleoyl-CoAs as primers but not eicosanoyl- or eicosenoyl-CoA. There were an anaerobic pathway and an aerobic desaturation one for the production of unsaturated fatty acids. Eicosapentaenoic acid seemed to be produced through the aerobic desaturation pathway and not through the anaerobic one, since the latter pathway produced *n*-7 type monoenoic fatty acids, which are different from eicosapentaenoic acid in the position of the double bond. The desaturase utilized an acyl-acyl carrier protein as a substrate, and this activity increased in the presence of ferredoxin and ferredoxin NADP$^+$ reductase. Thus, *Shewanella* sp., strain SCRC-2738, has novel characteristics as to both fatty acid chain elongation and desaturation systems.

Key words: *de novo* fatty acid synthesis, eicosapentaenoic acid, fatty acid chain elongation, fatty acid desaturation, marine bacterium.

Shewanella sp. strain SCRC-2738, which produces a large amount of eicosapentaenoic acid, 20:5, was isolated from the intestinal contents of Pacific mackerel (*1*). We previously reported the fatty acid and lipid compositions as well as the metabolism of exogenous fatty acids incorporated into cells (*2*). Most bacteria produce C16 and C18 saturated and monounsaturated fatty acids as major fatty acids, while animals and plants produce C20 and C22 polyunsaturated fatty acids in addition to C16 and C18 fatty acids. *Shewanella* is a unique bacterium with respect to the production of 20:5. Fatty acids are synthesized through three different systems, *de novo* synthesis, chain elongation and desaturation ones. Usually, the *de novo* synthesis system produces palmitic acid from acetyl-CoA, and the chain elongation one further increases the chain length of palmitic acid produced through *de novo* synthesis. Heretofore, three types of aerobic desaturase were known; acyl-CoA desaturase in mammals (*3, 4*), acyl-ACP desaturase in plants (*5*), and acyl-lipid desaturase in plants (*5, 6*), algae (*7*), and cyanobacteria (*8, 9*). Although there have been many reports on bacterial production of 20:5 (*1, 10-18*), the pathway remains to be solved. There was no information of the characteristics of the fatty acid *de novo* synthesis and chain elongation systems of *Shewanella*. Our previous study (*2*) showed that *Shewanella* does not possess the acyl-lipid desaturase present in plants (*5, 6*), and does not require 18:3(*n*-3) for the production of 20:5 like in mammals (*3*). Therefore, it is expected that *Shewanella* possesses novel fatty acid synthesis systems. In this study, we investigated the fatty acid synthesis systems of *Shewanella* to elucidate the synthetic pathway of 20:5.

[1] To whom correspondence should be addressed.

Abbreviations: ACP, acyl carrier protein; ASW, artificial seawater; FAS, fatty acid synthase; Fd, ferredoxin; FNR, ferredoxin NADP$^+$ reductase; 6:0-, hexanoyl-; 8:0-, octanoyl-; 10:0-, decanoyl-; 12:0-, lauroyl-; 14:0-, myristoyl-; 16:0-, palmitoyl-; 16:1(*n*-7)-, palmitoleoyl-; 18:0-, stearoyl-; 18:1(*n*-9)-, oleoyl-; 18:1(*n*-7)-, vaccenoyl-; 18:2(*n*-6)-, linoleoyl-; 18:3(*n*-3)-, linolenoyl-; 20:0-, eicosanoyl-; 20:1(*n*-9)-, eicosenoyl-; 14:0, myristic acid; 14:1, tetradecenoic acid; 16:0, palmitic acid; 16:1, hexadecenoic acid; 16:1(*n*-7), palmitoleic acid; 18:0, stearic acid; 18:1, octadecenoic acid; 18:1(*n*-9), oleic acid; 18:1(*n*-7), vaccenic acid; 20:0, eicosanoic acid; 20:1, eicosenoic acid; 22:0, docosanoic acid; 22:1, docosenoic acid; 20:5, 5,8,11,14,17-eicosapentaenoic acid; C16, C18, C20, and C26, 16, 18, 20, and 26 carbon atoms in a fatty acid, respectively.

MATERIALS AND METHODS

Chemicals—Acyl-CoAs, cerulenin, *Escherichia coli* ACP, spinach Fd, FNR (spinach leaf), and catalase (bovine liver) were purchased from Sigma Chemical (St. Louis, MO, USA); [1-^{14}C]acetyl-CoA (2.22 GBq/mmol), [2-^{14}C]malonyl-CoA (1.89 GBq/mmol), [1-^{14}C]16:0-CoA (2.05 GBq/mmol), and [1-^{14}C]18:1(*n*-9)-CoA (1.95 GBq/mmol) were from Amersham (Arlington, IL, USA); NADH and NADPH were from Tokyo Kasei (Tokyo); and the artificial seawater (ASW) was from Jamarin Laboratories (Osaka). All other reagents used were of analytical grade and commercially available.

Strains and Preparation of the Enzyme Solution—*Shewanella* sp. strain SCRC-2738 was cultivated in PY medium (*2*) at 18°C for 20 h on a rotary shaker. The cells were collected by centrifugation and washed with a half concentration of the artificial seawater (ASW). The washed cells were dispersed in 0.1 M potassium phosphate buffer (pH 7.0) to disrupt them, and the resultant solution was separated into a supernatant and a precipitate by centrifugation at 110,000×*g* at 4°C for 80 min. The precipitate was dispersed in ASW/0.2 M potassium phosphate buffer (pH 7.0) (1 : 1, v/v). *Vibrio* ABE-1, which mainly produces acyl-ACPs through the partial purified FAS, was kindly supplied by Prof. Hidetoshi Okuyama, Hokkaido University. *Vibrio* ABE-1 was cultivated at 15°C for 24 h in a medium (pH 7.0) containing 1% peptone, 1% meat extract, and 3.3% NaCl. The partial purified FAS of *Vibrio* ABE-1 was prepared according to the method of Morita *et al.* (*19*).

Reaction Mixture for Fatty Acid Synthesis—The standard reaction mixture for fatty acid synthesis consisted of 0.1 M potassium phosphate buffer (pH 7.0), 0.25 ml of ASW, 52 μM [2-^{14}C]malonyl-CoA, 28 μM acyl-CoA indicated in each experiment, 0.1 mg of ACP, 1.7 mM NADPH, 1.7 mM NADH, and 0.1 mg protein of the enzyme solution, in a total volume of 0.5 ml. In some experiments, [1-^{14}C]acetyl-CoA was used instead of [2-^{14}C]malonyl-CoA. The reaction was started by adding the enzyme solution and carried out at 25°C for 30–60 min.

Preparation of [^{14}C]Acyl-ACPs with FAS of Vibrio ABE-1—To prepare [^{14}C]acyl-ACPs, we used the partial purified *Vibrio* ABE-1 fatty acid synthase. The reaction mixture comprised 80 mM potassium phosphate buffer (pH 7.0), 30 μM acetyl-CoA, 0.21 mM [2-^{14}C]malonyl-CoA, 0.15 mg of ACP, 0.15 mM NADPH, 0.15 mM NADH, and 0.51 mg protein of the partially purified enzyme, in a total volume of 0.53 ml. The reaction was started by adding the enzyme solution and carried out at 20°C for 60 min. After the reaction, the mixture was used for the following desaturation assay.

Reaction Mixture for Desaturation—The standard reaction mixture for desaturase consisted of 0.1 M potassium phosphate buffer (pH 7.0), 0.22 ml of ASW, 0.25 ml of the above reaction mixture for *Vibrio* ABE-1 or 50 μM [1-^{14}C]16:0-CoA, 1.5 mM NADPH, 1.5 mM NADH, 0.12 mg of Fd, 0.33 unit of FNR, and 0.8 mg protein of the enzyme solution prepared from *Shewanella*, in a total volume of 0.5 ml. The reaction was started by adding the enzyme solution and carried out at 25°C for 120 min.

Analysis of FAS Product—In order to determine that the product of *Shewanella* FAS is an ACP derivative, the reaction was stopped by adding the same volume of a dye solution comprising 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenyl blue in 0.0625 M Tris·HCl (pH 6.8). The mixture was heated at 100°C for 3 min and then subjected to SDS-PAGE. Electrophoresis was conducted on a 10–20% gradient SDS-polyacrylamide gel, the buffer system consisting of 1.44% glycine, 0.1% SDS, and 0.3% Tris, at a constant current, 30 mA, for 60 min. The gel was dried and visualized by autoradiography.

Analysis of the Fatty Acids Produced through Shewanella FAS Reactions—To identify the fatty acids produced by *Shewanella* FAS, the reaction mixture was lyophilized and then heated at 95°C for 60 min in an 8% HCl-methanolic solution. The fatty acid methyl esters were extracted with *n*-hexane, and then separated on silica gel-TLC plates (Merck Art 5721) developed with *n*-hexane/diethyl ether (80 : 20, v/v) or on silver nitrate-immersed silica gel-TLC plates developed with *n*-hexane/diethyl ether (95 : 5, v/v, or 83 : 17, v/v). The fatty acid methyl esters on the silica gel-TLC and silver nitrate-immersed silica gel-TLC plates were visualized with 0.02% primlin in 80% acetone and 0.2% dichlorofluorescein in 95% ethanol, respectively. The radioactivity on the TLC plates was located with a radioanalytic imaging system (AMBIS Systems, CA, USA). The radioactive spots were scraped off the plates, and fatty acid methyl esters were extracted with chloroform/methanol (2 : 1, v/v) and then analyzed, using a Shimadzu radio gas chromatograph GC-6A equipped with a flame ionization detector and a proportional counter, on a column packed with Shinchrom E-71 (3 mm×2 m), according to the method described in the previous report (*2*). The column temperature was kept at 200°C for the first 5 min and then increased to 250°C at 4°C/min.

Other Analytical Methods—The concentrations of protein were determined with a protein assay kit obtained from Bio-Rad (Richmond, CA, USA). The radioactivity was measured with a Packard liquid scintillation counter, model LS 4320.

RESULTS

Fatty Acid Synthesis by the Enzyme Preparation of Shewanella—Fatty acids produced by the enzyme preparation were analyzed to characterize the FAS system of *Shewanella* (Fig. 1). The supernatant produced 14:0, 16:0, and 16:1 from [1-^{14}C]acetyl-CoA and malonyl-CoA (Fig. 1A). However, no fatty acid was produced from [1-^{14}C]-acetyl-CoA and malonyl-CoA with the precipitate (data not shown). The supernatant also produced 18:0 from [1-^{14}C]-16:0-CoA and malonyl-CoA (Fig. 1B), and 20:0 from 18:0-CoA and [2-^{14}C]malonyl-CoA (Fig. 1D). The production of 14:0, 16:0, and 16:1 can also be observed in Fig. 1D. This might be due to utilization of endogenous acetyl-CoA present in the enzyme preparation instead of exogenous 18:0-CoA as a primer. The production of 20:0 was negligible from 18:0-CoA and [1-^{14}C]acetyl-CoA with the supernatant (data not shown). It was shown that *Shewanella* does not possess a fatty acid synthesis system which utilizes acetyl-CoA as a condensing unit. When the reaction was carried out with [1-^{14}C]16:0-CoA and malonyl-CoA in the presence of the precipitate (Fig. 1C), one radioactive peak other than that of 16:0 was detected. Both the R_f value on silver nitrate-immersed silica gel-TLC and the retention time on radio GLC of this peak material were in agreement with those of *trans*-2-hexadecenoic acid, which is produced by acyl-CoA dehydrogenase, which catalyzes the first step of the β-oxidation cycle (data not shown). These results suggested that the fatty acid synthetic activities as to both acetyl-CoA to C16 and 16:0-CoA to 20:0 are located in the supernatant.

Product of the FAS Reaction—E. coli FAS produces acyl-ACP derivatives (*20*), and the FASs from yeast (*21*) and coryneform bacteria (*22*) produce acyl-CoA derivatives. On the other hand, mammalian FASs produce free fatty acids. To identify the final product of *Shewanella* FAS, the reaction mixture was subjected to SDS-PAGE (Fig. 2). When the reaction was carried out with [2-^{14}C]-

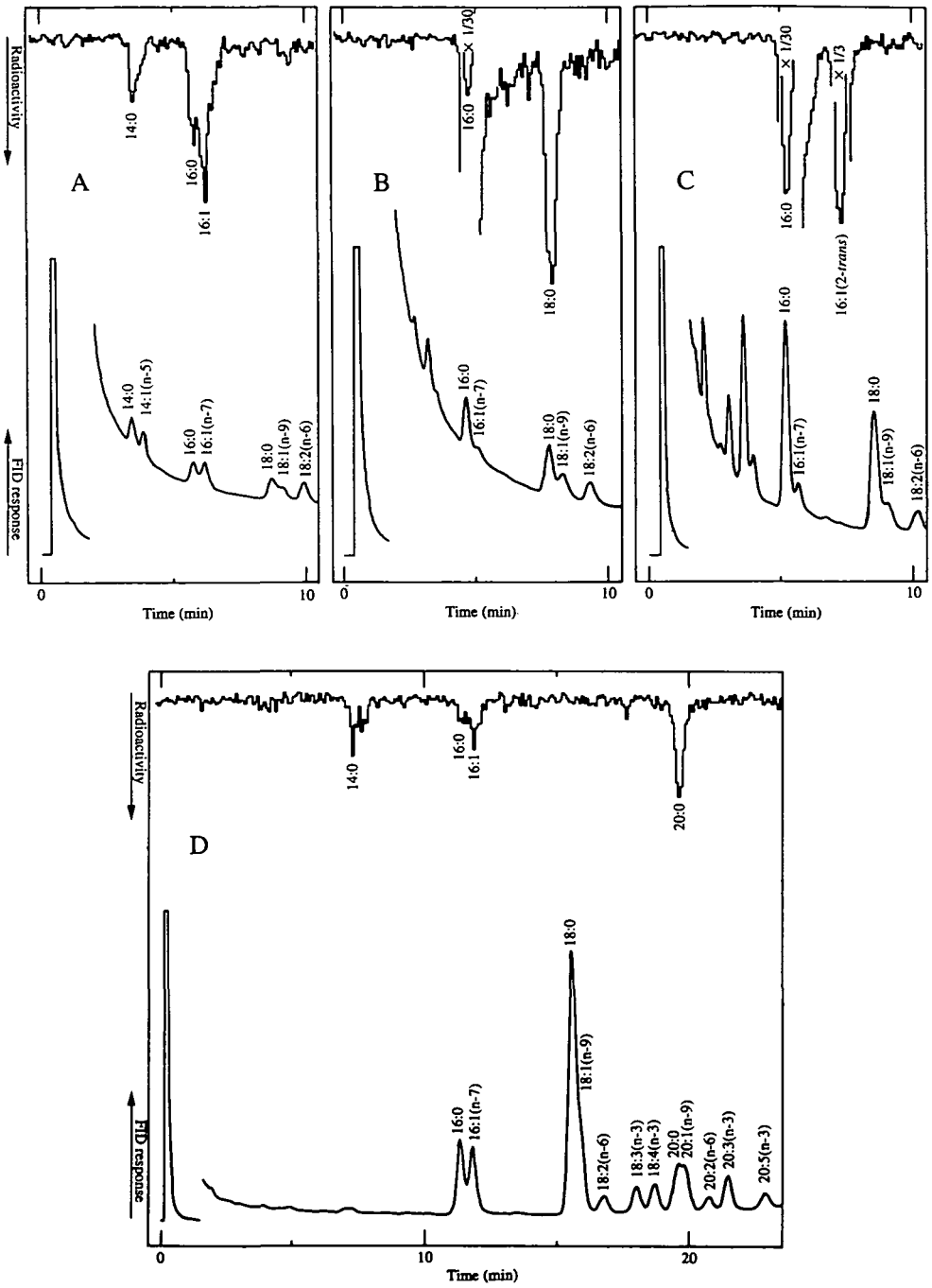

Fig. 1. **Radio gas chromatograms of the fatty acids produced with the enzyme preparation.** The reaction mixture consisted of 0.1 M potassium phosphate buffer (pH 7.0), 0.25 ml of ASW, 36 μM [1-^{14}C]acetyl-CoA, [1-^{14}C]16:0-CoA, or 18:0-CoA indicated in each experiment, 0.1 mM malonyl-CoA or 52 μM [2-^{14}C]malonyl-CoA, 1.7 mM NADPH, 1.7 mM NADH, 0.3 mg of ACP, and the precipitate (0.8 mg protein) or supernatant (0.25 mg protein), in a total volume of 0.5 ml. The reactions were carried out at 25°C for 60 min. A: Fatty acids synthesized from [1-^{14}C]acetyl-CoA with the supernatant. B: Fatty acids synthesized from [1-^{14}C]16:0-CoA with the supernatant. C: Fatty acids synthesized from [1-^{14}C]16:0-CoA with the precipitate. D: Fatty acids synthesized from 18:0-CoA and [2-^{14}C]malonyl-CoA with the supernatant. The radio GLC analyses in A, B and C were carried out as described under "MATERIALS AND METHODS." In D, the column temperature for radio GLC was kept at 160°C for the first 2 min and then increased to 248°C at 4°C/min.

malonyl-CoA and acetyl-CoA as primers, two bands were detected at *ca.* 14 kDa (lane 2). This profile is the same as that of *Vibrio* ABE-1 FAS (lane 1), which produces acyl-ACPs as final products (*19*). The lower band was major for both the FASs and seemed to be an acyl-ACP with respect to the molecular weight, although the identities of the upper band materials are currently not known. The product of the reaction with malonyl-CoA and [1-^{14}C]18:1(*n*-9)-CoA as a primer was also detected at *ca.* 14 kDa (lane 5). This product was considered to be [^{14}C]20:1-ACP and was not detected in the absence of malonyl-CoA (lane 3). No product was detected at *ca.* 14 kDa in the control reactions in both the absence and presence of malonyl-CoA, which were carried out with the heated enzyme solution (lanes 4 and 6, respectively). The fatty acid compositions of these products were determined by means of the following examination.

Fatty Acids Synthesized by the Shewanella FAS Preparation—Table I shows the amounts of fatty acids synthesized from [2-^{14}C]malonyl-CoA and various acyl-CoAs as primers. The production of a small amount of fatty acids was observed in the absence of an exogenous primer. This might be due to endogenous acetyl-CoA present in the enzyme preparation, as shown in Fig. 1D. When acetyl-CoA was used as a primer, 16:1 was the major product, and comparable amounts of saturated fatty acids (14:0 and 16:

0) were also produced. The product pattern was similar to that for acetyl-CoA when 6:0- or 8:0-CoA was used as a primer. On the other hand, when 10:0-, 12:0-, or 14:0-CoA was used as a primer, the products were exclusively saturated fatty acids, and 16:1 was almost undetectable. The production of 16:1 implies that *Shewanella* has an anaerobic desaturation pathway involving β,γ-dehydration of a β-hydroxyacyl intermediate (*20*). Because the amount of 16:1 produced in the reaction involving an acyl-CoA with a longer-chain than C10 was negligible, it was implied that the β,γ-dehydrase is specific for β-hydroxydecanoate. When a longer-chain acyl-CoA than 14:0-CoA was used as a primer, the fatty acid produced was C2 units longer than the primer. The production of 22:0 and 22:1 from 20:0- and 20:1(n-9)-CoA was negligible.

These results suggested that *Shewanella* possesses two different types of FAS system. One is a *de novo* synthesis system, which produces C16 fatty acids from acetyl-CoA and malonyl-CoA, and the other is a chain elongation system, which produces C20 fatty acids from C16 fatty acids. It was also shown that there was an anaerobic pathway for the production of n-7 type monoenoic acids like in *E. coli* (*19*).

Characteristics of the Shewanella FAS Systems—Table II shows the effects of ACP, NADPH, and NADH on the incorporation of [2-^{14}C]malonyl-CoA into fatty acids. In the experiment with acetyl-CoA as a primer, we could estimate the activity of *de novo* synthesis. *De novo* synthesis absolutely required NADPH and ACP. In the absence of NADH, the amount of fatty acid synthesized was 27% of that with the complete system. In the experiment with 18:0-CoA as a primer, we could determine the activity of chain elongation. The requirements for chain elongation are similar to those for *de novo* synthesis.

Figure 3 shows the effects of cerulenin on the activities of the *de novo* synthesis and chain elongation systems. The *de novo* synthesis activity decreased drastically with an increase in the cerulenin concentration, and 50 μM cerulenin completely inhibited the activity. On the other hand, chain elongation was much less sensitive to cerulenin. With 50 μM cerulenin, the chain elongation activity remaining was still about 50%. As described previously, the enzyme preparation used in this experiment probably contained endogenous acetyl-CoA. About half the amount of fatty acids was produced through *de novo* synthesis from [2-^{14}C]-

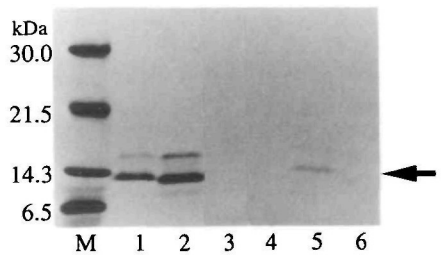

Fig. 2. **SDS-PAGE of the reaction products of FAS.** Lane M: molecular weight standards. Products from [2-^{14}C]malonyl-CoA and acetyl-CoA with FASs of *Vibrio* ABE-1 (lane 1) and *Shewanella* sp. (lane 2). Products of *Shewanella* FAS from [1-^{14}C]18:1(n-9)-CoA in the absence (lanes 3 and 4) and presence (lanes 5 and 6) of malonyl-CoA; lanes 3 and 5, native enzyme solution; lanes 4 and 6, heated (100°C, 3 min) enzyme solution.

TABLE II. **Effects of ACP, NADPH, and NADH on the activities of *de novo* synthesis and chain elongation.** The amount of [2-^{14}C]malonyl-CoA incorporated was determined for 14:0, 16:0, and 16:1 in the reactions with acetyl-CoA (*de novo* synthesis), and for 20:0 in the reactions with 18:0-CoA (chain elongation) by the same method as described in Table I.

Reaction mixture	Amount of [2-^{14}C]malonyl-CoA incorporated into fatty acids (nmol)
Complete system (primer, acetyl-CoA)	4.7[a]
−NADH	1.3[a]
−NADPH	nd
−NADPH, −NADH	nd
−ACP	nd
Complete system (primer, 18:0-CoA)	0.8[b]
−NADH	0.6[b]
−NADPH	0.1[b]
−NADPH, −NADH	nd
−ACP	nd

[a]Total of 14:0, 16:0, and 16:1. [b]20:0. nd: not detected.

TABLE I. **Fatty acids synthesized from [2-^{14}C]malonyl-CoA.** The amount of [2-^{14}C]malonyl-CoA incorporated into each fatty acid was determined by radio GLC and liquid scintillation counting as described under "MATERIALS AND METHODS."

Primer	Amount of [2-^{14}C]malonyl-CoA incorporated into (nmol)										
	14:0[a]	16:0	16:1	18:0	18:1	20:0	20:1	20:2	20:3	22:0	22:1
None (control)	0.4	0.3	0.4	nd	nd	nd	nd	nd	nd	nd	nd
Acetyl-CoA	1.6	1.0	2.6	nd	nd	nd	nd	nd	nd	nd	nd
6:0-CoA	3.0	0.9	2.7	nd	0.2	nd	nd	nd	nd	nd	nd
8:0-CoA	2.6	1.2	2.9	nd	0.3	nd	nd	nd	nd	nd	nd
10:0-CoA	2.9	2.6	0.4	nd	0.1	nd	nd	nd	nd	nd	nd
12:0-CoA	0.7	1.8	nd	nd	nd	nd	nd	nd	nd	nd	nd
14:0-CoA	0.2	1.3	nd	0.1	nd	nd	nd	nd	nd	nd	nd
16:0-CoA	0.3	nd	0.2	0.8	nd	nd	nd	nd	nd	nd	nd
18:0-CoA	0.3	0.1	0.3	nd	nd	0.8	nd	nd	nd	nd	nd
20:0-CoA	0.4	0.2	0.3	nd	nd	nd	nd	nd	nd	0.1	nd
16:1(n-7)-CoA	0.1	0.1	0.2	nd	1.4	nd	0.1	nd	nd	nd	nd
18:1(n-9)-CoA	0.3	nd	0.2	nd	nd	nd	0.7	nd	nd	nd	nd
18:2(n-6)-CoA	0.4	nd	0.2	nd	nd	nd	nd	0.4	nd	nd	nd
18:3(n-3)-CoA	0.4	nd	0.1	nd	nd	nd	nd	nd	0.4	nd	nd
20:1(n-9)-CoA	0.4	0.2	0.3	nd	0.1	nd	nd	nd	nd	nd	0.2

[a]Under the conditions adopted, it was difficult to separate 14:0 and 14:1. Judging from the radiochromatogram, 14:0 was a major product and 14:1 was a minor one, if any.

malonyl-CoA and endogenous acetyl-CoA instead of added 18:0-CoA (Table I). Therefore, it is likely that cerulenin mainly inhibited the *de novo* synthesis involving endogenous acetyl-CoA and that cerulenin has little effect on chain elongation involving added 18:0-CoA.

Characteristics of the Desaturation System—The unsaturated fatty acids produced by the enzyme prepared from the precipitate were analyzed by silver nitrate-immersed silica gel-TLC and radio GLC. Radioactivity was detected in the dienoic acid fraction when [^{14}C]acyl-ACPs were used as substrates. The radioactivity in the dienoic acid fraction was negligible in the absence of Fd and FNR (Fig. 4, lane 2). The [^{14}C]acyl-ACPs prepared with the partial purified FAS of *Vibrio* ABE-1 included saturated and monoenoic acids, but not dienoic acids (*19*). These dienoic acids were not detected in the control reaction carried out with the heated enzyme solution (Fig. 4, lane 3). Therefore, it is considered that these dienoic acids were produced by the *Shewanella* enzyme preparation. The dienoic acids contained 16:2, 18:2, 20:2, and 22:2, as judged from their retention times on radio GLC (Fig. 5). The radioactivity in the other polyenoic acid fractions was not due to any fatty acids, because no radioactivity was detected on radio GLC of the extracts of the fractions.

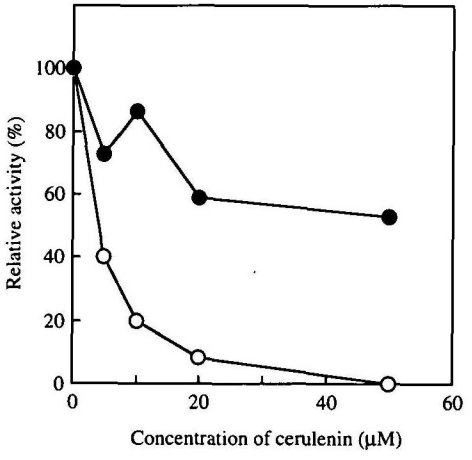

Fig. 3. **Effects of cerulenin on the activities of *de novo* synthesis and chain elongation.** The reaction was carried out with acetyl-CoA (*de novo* synthesis, ○) or 18:0-CoA (chain elongation, ●) as a primer, in the presence of the indicated concentrations of cerulenin. The amount of [2-^{14}C]malonyl-CoA incorporated into fatty acids was measured.

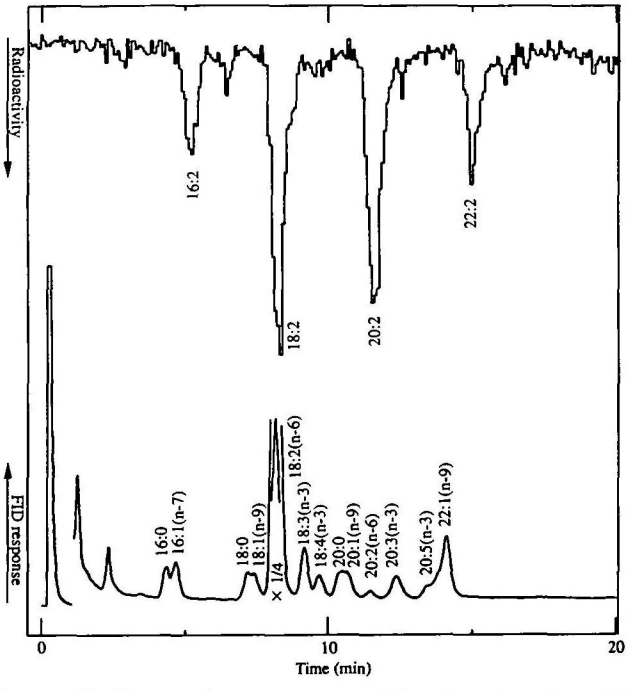

Fig. 5. **Radio gas chromatograms of the dienoic fatty acids produced through the desaturation reaction with [^{14}C]acyl-ACPs.** The radioactive dienoic acid fraction of lane 1 on silver nitrate TLC (Fig. 4) was scraped off and subjected to radio GLC.

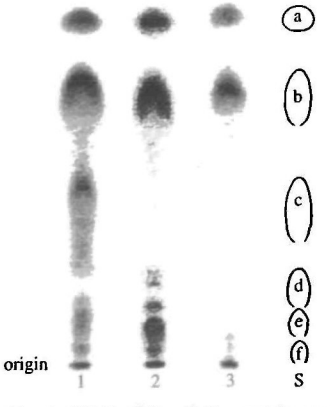

Fig. 4. **Silver nitrate TLC of the fatty acids produced through the desaturation reaction with [^{14}C]acyl-ACPs as substrates.** The TLC plate was developed three times with *n*-hexane/diethyl ether (87 : 13, v/v). Lane 1, fatty acids produced under the standard reaction conditions; lane 2, fatty acids produced in the absence of Fd and FNR; lane 3, the precipitate was heated at 100°C for 3 min before use under the standard reaction conditions. Lane S, standard fatty acids; a, b, c, d, e, and f, 18:0, 18:1(*n*-9), 18:2(*n*-6), 18:3(*n*-3), 18:4(*n*-3), and 20:5(*n*-3), respectively.

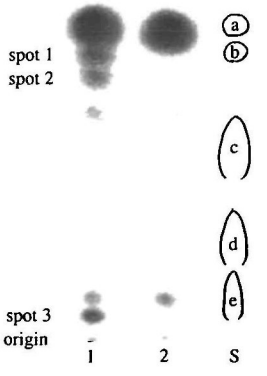

Fig. 6. **Silver nitrate TLC of the fatty acids produced through the desaturation reaction with [1-^{14}C]16:0-CoA as a substrate.** The standard reaction mixture for desaturation was used. The TLC plate was developed twice with *n*-hexane/diethyl ether (95 : 5, v/v). Lane 1, fatty acids produced from [1-^{14}C]16:0-CoA with the precipitate; lane 2, the precipitate was heated at 100°C for 3 min before the reaction. Lane S, standard fatty acids; a, b, c, d, and e, 18:0, 16:1(2-*trans*), 18:1(*n*-9), 18:2(*n*-6), and 18:3(*n*-3), respectively.

When the reaction was carried out with [1-^{14}C]16:0-CoA as a substrate in order to examine the activity of acyl-CoA desaturase, three major products, spots 1, 2, and 3, were detected on silver nitrate-immersed silica gel-TLC (Fig. 6, lane 1). The largest product was spot 1, which was confirmed to be *trans*-2-hexadecenoic acid by both the R_f value on TLC and the retention time on radio GLC (data not shown). While the spot 2 material was very similar to 16:1(n-7) with respect to the retention time on radio GLC (data not shown), the spot was between those of saturated and monoenoic acids on TLC. The spot 3 material was not a fatty acid, because no radioactivity was detected on radio GLC. The identities of the spot 2 and 3 materials are currently not known. The desaturase products were negligible. These results implied that acyl-CoA was preferred as a substrate for β-oxidation rather than desaturation.

DISCUSSION

The fatty acid synthesis system of *Shewanella* sp. strain SCRC-2738 was examined. Since cell lysate was separated into a supernatant and a precipitate by centrifugation at 110,000$\times g$, the supernatant and precipitate contained the cytosol and membrane fractions of the cells, respectively (*23-25*). Therefore, both the *de novo* synthesis system and the chain elongation system of *Shewanella* are in the cytosol.

The *de novo* synthesis of *Shewanella* absolutely required ACP and NADPH, with C16-ACPs as the final products. The chain elongation system of *Shewanella* also absolutely required ACP and NADPH, and produced up to C20 from C16. Since both activities increased with the coexistence of NADH, NADH seems to be highly specific for either β-ketoacyl-ACP reductase or enoyl-ACP reductase. The *de novo* synthesis was highly sensitive to cerulenin. It is known that the *de novo* synthesis system of *E. coli* exhibits similar cofactor requirements (*20*), and that β-ketoacyl-ACP synthases I and II of *E. coli* are sensitive to cerulenin but that β-ketoacyl-ACP synthase III is not (*26*). Although it is not known whether or not *Shewanella* possesses an enzyme like β-ketoacyl-ACP synthase III of *E. coli*, the *de novo* synthesis system of *Shewanella* is similar to that of *E. coli*. However, the chain elongation system of *E. coli* differs from that of *Shewanella* because *E. coli* produces until C18 but not C20 from C16. In higher plants, while C18 is produced from C16 by an ACP-dependent chain elongation system (*27, 28*), C20 is produced from C18 by a CoA-dependent one (*6*). The chain elongation system of animals is CoA-dependent and is located in the microsome membrane (*29*). Compared with the chain elongation systems of other organisms, as to characteristics and location, the system of *Shewanella* seems to be a novel one. *Shewanella* does not possess an acetyl-CoA dependent chain elongation system like those detected in the mitochondria of mammalian liver and brain (*30*), and *Euglena gracilis* (*31*), because the condensation of acetyl-CoA to 18:0-CoA was negligible.

It was shown that *Shewanella* does not possess an acyl-lipid desaturase in our previous study (*2*). *Shewanella* possesses an anaerobic pathway that produces only n-7 type monoenoic acid. The n-7 type monoenoic acid is unable to be a precursor of 20:5, because the double bond position of the acid is different from that of 20:5. In this study, we examined the activities of acyl-ACP desaturase and acyl-CoA desaturase. The desaturation system showed the following characteristics: (1) substrate specificity for acyl-ACP, and (2) requirement of Fd and FNR. We speculate that this acyl-ACP dependent desaturation system participates in the synthesis of 20:5 from the products of the chain elongation system, such as 18:0-ACP and 20:0-ACP, *in vivo*. It is not known which position of the carbon chain of a saturated acid is desaturated at first. Since acyl-ACP desaturase, which is located in the stroma of chloroplasts of higher plants, only desaturates 18:0-ACP to 18:1(n-9)-ACP (*5*), it is different from the desaturation system of *Shewanella*. With respect to the substrate type, the desaturation systems of other bacteria (*20, 24, 32*), plants (*5, 6*), algae (*7*), cyanobacteria (*8, 9*), and mammals (*3, 4*) are different from that of *Shewanella*. Therefore, the desaturation system of *Shewanella* seems to be a novel type.

According to the results, we propose the fatty acid synthesis pathway of *Shewanella* (Fig. 7). There are *de novo* synthesis, chain elongation and desaturation systems. There are an anaerobic pathway and an aerobic desaturation one for the production of unsaturated fatty acids. The anaerobic pathway, which produces n-7 type monounsaturated acid, does not contribute to the synthesis of 20:5. While the *de novo* synthesis system and anaerobic desaturation pathway are similar to those of *E. coli*, the chain elongation and aerobic desaturation systems are unique ones.

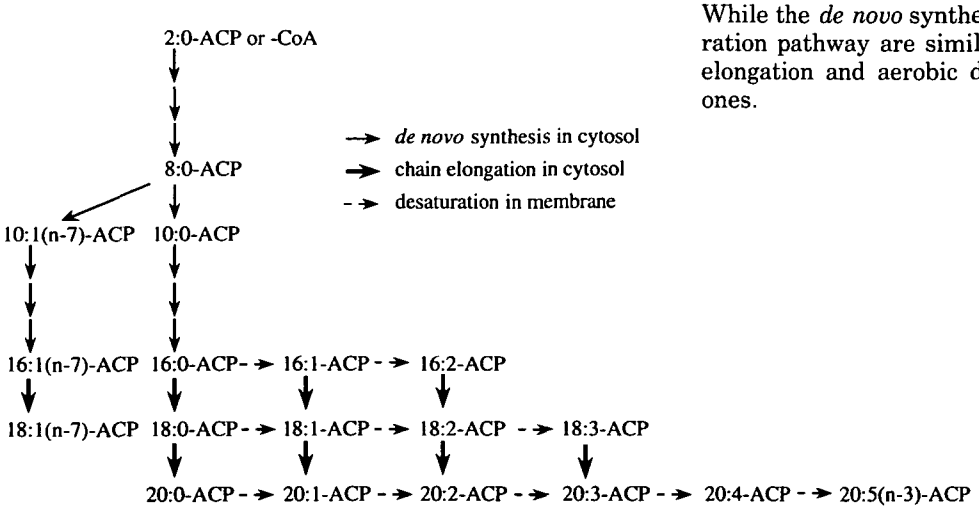

Fig. 7. **Possible pathways for 20:5 synthesis in *Shewanella* sp. strain SCRC-2738.**

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