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Δ9 Acyl-Lipid Desaturases of Cyanobacteria

MOLECULAR CLONING AND SUBSTRATE SPECIFICITIES IN TERMS OF FATTY ACIDS, *sn*-POSITIONS, AND POLAR HEAD GROUPS*

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In cyanobacteria, the biosynthesis of unsaturated fatty acids is initiated by Δ9 acyl-lipid desaturase which introduces the first double bond at the Δ9 position of a saturated fatty acid that has been esterified to a glycerolipid. We have cloned genes, designated *desC*, for Δ9 acyl-lipid desaturases from two cyanobacteria, namely *Anabaena variabilis* and *Synechocystis* sp. PCC 6803. These desaturases, when expressed in *Escherichia coli*, desaturated stearic acid to yield oleic acid at the C-1 positions of phosphatidylethanolamine and phosphatidylglycerol, but did not desaturate palmitic acid, palmitoleic acid, and *cis*-vaccenic acid. These results indicate that the Δ9 acyl-lipid desaturases are specific to stearic acid esterified at the C-1 position of a glycerolipid and are nonspecific with respect to the polar head group of the glycerolipid. The deduced amino acid sequences of the Δ9 acyl-lipid desaturases are similar in part to those of stearoyl-CoA desaturases of the rat, the mouse, and *Saccharomyces cerevisiae*, but not to those of acyl-(acyl-carrier-protein) desaturases of higher plants.

In higher plants and cyanobacteria, fatty acids are desaturated while they are esterified to glycerolipids (Harwood 1988; Jaworski, 1987; Sato *et al.*, 1986). The enzymes that catalyze this type of desaturation reaction are known as acyl-lipid desaturases and are bound to membranes. In addition to acyl-lipid desaturases, higher plants contain acyl-ACP[1] desaturases, which introduce a double bond only into saturated fatty acids that are bound to ACP (McKeon and Stumpf, 1982; Stumpf, 1981; Cahoon and Ohlrogge, 1994). These latter desaturases are soluble in the stroma of the chloroplast. In animals and fungi, fatty acids are desaturated in a CoA-bound form (Holloway, 1983), and the enzymes that catalyze the reaction are known as acyl-CoA desaturases and are bound to the endoplasmic reticulum (Strittmatter *et al.*, 1974).

In the cyanobacterium *Synechocystis* sp. PCC 6803, unsaturated fatty acids are synthesized via sequential desaturation of 18:0 at the C-1 position of glycerolipids to 18:1(9), 18:1(9, 12), 18:3(6, 9, 12), and 18:4(6, 9, 12, 15). These desaturation reactions involve four distinct acyl-lipid desaturases, each of which is strictly specific to its own position in the fatty-acyl chain at which a double bond is introduced (Murata *et al.* 1992; Wada and Murata, 1989, 1990).

Genes for the Δ12 acyl-lipid desaturase (*desA*; Wada *et al.* 1990), the ω3 acyl-lipid desaturase (*desB*; Sakamoto *et al.*, 1994a), and the Δ6 acyl-lipid desaturase (Reddy *et al.*, 1993) have been cloned from *Synechocystis* sp. PCC 6803. The *desA* genes have also been isolated from three other strains of cyanobacteria, namely *Synechocystis* sp. PCC 6714, *Synechococcus* sp. PCC 7002, and *Anabaena variabilis* (Sakamoto *et al.*, 1994b). Genetic manipulation of the *desA* gene of *Synechocystis* sp. PCC 6803 demonstrated that the fatty acid unsaturation is essential for the low temperature tolerance of cyanobacteria (Gombos *et al.* 1992, 1994; Wada *et al.* 1990, 1994). In higher plants, cDNAs for ω3 acyl-lipid desaturases of the endoplasmic reticulum (Arondel *et al.*, 1992; Yadav *et al.*, 1993) and chloroplasts (Iba *et al.*, 1993; Yadav *et al.*, 1993) have been isolated from *Arabidopsis thaliana*. A gene for the Δ12 acyl-lipid desaturase of the endoplasmic reticulum has also been isolated from *A. thaliana* (Okuley *et al.*, 1994). However, there are no reports of the molecular and biochemical characterization of the Δ9 acyl-lipid desaturases of either cyanobacteria or higher plants.

EXPERIMENTAL PROCEDURES

Organisms and Culture Conditions—*Synechocystis* sp. PCC 6803 from the Pasteur Culture Collection and *A. variabilis* strain M-3 from the Algal Culture Collection of the Institute of Applied Microbiology, University of Tokyo, were grown photoautotrophically at 34 °C, as described previously (Wada and Murata, 1989). Transformed cells of *Escherichia coli* strain BL21(DE3)pLysS (Studier *et al.*, 1990) were grown at 37 °C in M9 medium supplemented with 1 mM $MgSO_4$, 0.2% glucose, 0.5 μg ml^{-1} vitamin B_1, 0.1% casamino acids, 50 μg ml^{-1} ampicillin, 20 μg ml^{-1} chloramphenicol, 10 μM $FeCl_3$, and 0.1 mM sodium stearate (S0081, Tokyokasei, Tokyo, Japan).

Cloning of the desC Gene of A. variabilis—The *desA* gene of *A. variabilis* had been cloned previously as a 7-kbp *Eco*RI fragment from the genomic DNA of *A. variabilis* (Fig. 1*A*; Sakamoto *et al.*, 1994b). We found an open reading frame in the 5′-upstream region of the *desA* gene. As shown below, this open reading frame encoded a Δ9 acyl-lipid desaturase, and the gene was designated "*desC*."

Cloning of the desC Gene of Synechocystis—A genomic DNA library of the *desA*-Δ mutant of *Synechocystis* sp. PCC 6803 was constructed with a phage vector, λ*DASH* II (Stratagene, La Jolla, CA), as described previously (Sakamoto *et al.*, 1994a). Approximately 2,500 plaques of the

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D16547. (*desC gene of Synechocystis* sp. PCC 6803) and D14581 (desC gene of *A. variabilis*).
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[1] The abbreviations used are: ACP, acyl-carrier protein; CL, cardiolipin; IPTG, isopropyl-1-thio-β-D-galactoside; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; *X*:*Y*(*Z*), fatty acid containing *X* carbon atoms with *Y* double bonds in the *cis* configuration at position *Z* counted from the carboxyl terminus; kbp, kilobase pair(s).

recombinant phages were screened with a 0.75-kbp probe derived from the *desC* gene of *A. variabilis* (Fig. 1*A*). Hybridization was performed under the conditions of low stringency as described previously (Sakamoto *et al.*, 1994a). A total of 22 positive clones was obtained.

One of the positive clones contained a *Hin*dIII DNA fragment of 6 kbp in its insert. This *Hin*dIII fragment was subcloned into the *Hin*dIII site of pBluescript II KS(+) (Stratagene). The resultant plasmid was designated pBluescript/H6. Then 1,275 base pairs of the nucleotide sequence of the 6-kbp insert, which hybridized the 0.75-kbp probe, was determined by the dideoxy chain termination method (Sanger *et al.*, 1977) using a *Bca*BEST dideoxy sequencing kit (Takara, Kyoto, Japan).

Analysis of the Deduced Amino Acid Sequence—A search for proteins with amino acid sequences similar to the deduced amino acid sequences encoded by the *desC* genes of *A. variabilis* and *Synechocystis* sp. PCC 6803 was performed using the BLAST algorithm (Altschul *et al.*, 1990) at the National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, (Bethesda, MD). The alignment of amino acid sequences was performed using the molecular evolutionary analysis system for DNA and amino acid sequences, ODEN, at the National Institute of Genetics (Mishima, Japan).

Expression of the desC Gene in E. coli—The *desC* gene of *A. variabilis* was amplified by polymerase chain reaction with the λDNA clone for the *desC* gene of *A. variabilis* as the template and with two synthesized polynucleotides, 5′-GGAAGCTTATGACTAGTGCTACTTCAACTAAAC-CTCAAATC and 3′-ATCGTCTGTTTTCGTCATTCGAAGG, as the primers. The resultant product of 0.8 kbp was subcloned into the *Hin*dIII site of pBluescript II(Ks$^+$) and the resultant plasmid was designated pBluescript/*desC*(A). The nucleotide sequence of the insert in pBluescript/*desC*(A) was determined to confirm the presence of the gene. Then pBluescript/*desC*(A) was digested with *Spe*I and the resultant fragment of 0.8 kbp, containing the coding region, was subcloned into the *Nhe*I site of pET3a, a T7 RNA polymerase-regulated expression plasmid (Studier *et al.*, 1990). The resultant plasmid was designated pET3a/*desC*(A). As a consequence of these manipulations, the amino-terminal sequence of the gene product was changed from M-T-I- to M-A-S-. The plasmid was introduced into *E. coli* strain BL21(DE3)pLysS. Wild-type *E. coli* does not contain any Δ9 desaturase (Holloway, 1983).

The 5′-half of the coding region of the *desC* gene of *Synechocystis* sp. PCC 6803 was amplified by polymerase chain reaction with the λDNA clone for the *desC* gene of *Synechocystis* sp. PCC 6803 as the template and with two synthesized polynucleotides, 5′-GGTCTAGAATGACT-AGTCCATTAAACATTGAATACCTATAT and 3′-CTGTGGTGGGGCC-TAGGGGT, as the primers (Fig. 1*C*). The product of 0.5 kbp was digested with *Xba*I and *Bam*HI, and the resultant fragment was subcloned into the *Xba*I/*Bam*HI site of pBluescript/H6 which contained the 3′-half of the coding region of the *desC* gene. The resultant plasmid was designated pBluescript/*desC*(S). The nucleotide sequence of the amplified region was confirmed by the standard method. Then pBluescript/*desC*(S) was digested with *Spe*I, and the resultant fragment of 1.1 kbp, containing the coding region, was subcloned into the *Nhe*I site of pET3a to yield pET3a/*desC*(S) (Fig. 1*C*). The amino-terminal sequence of the product of the *desC* gene of *Synechocystis* sp. PCC 6803 was changed from M-L-N- to M-A-S-. The plasmid was introduced into *E. coli* strain BL21(DE3)pLysS.

Analysis of Fatty Acids in E. coli Cells—*E. coli* cells transformed with pET3a, pET3a/*desC*(A), or pET3a/*desC*(S) were grown to an optical density at 600 nm (OD_{600}) of 0.6. Then IPTG was added to a final concentration of 1 mM. After the culture had been incubated for a further hour, the cells were collected by centrifugation and washed with 1.2% NaCl. Lipids were extracted from the collected cells by the method of Bligh and Dyer (1959). PE, PG, and CL were separated by thin-layer chromatography on precoated silica gel plates (5721; Merck, Darmstadt, Germany) with a mixture of $CHCl_3$, CH_3OH, and CH_3COOH (65:25:10, v/v) as the mobile phase. Then they were subjected to methanolysis in 5% (w/w) HCl/methanol at 85 °C for 2.5 h. To each of samples, 300 nmol of 20:0 was added as an internal standard to quantify the fatty acid methyl esters. The resultant methyl esters were analyzed by gas chromatography as described previously (Wada and Murata, 1989). The distribution of fatty acids in the glycerol moiety of PE and PG was analyzed by selective hydrolysis by a lipase from *Rhizopus delemar* (Boehringer Mannheim) as described by Fischer *et al.* (1973).

RESULTS

The desC Gene of A. variabilis—The nucleotide sequence of part of the 7-kbp DNA fragment that contained the *desA* gene of *A. variabilis* revealed a novel open reading frame in the 5′-upstream region of the *desA* gene (Fig. 1*A*). This open reading frame encodes a polypeptide of 272 amino acid residues. The deduced amino acid sequence of the polypeptide (Fig. 2) resembles those of 18:0-CoA desaturases from the rat (31%), the mouse (31%), and *S. cerevisiae* (29%). We postulated that the open reading frame encodes a Δ9 acyl-lipid desaturase of *A. variabilis*. The gene was therefore designated *desC*.

The desC Gene of Synechocystis sp. PCC 6803—The genomic DNA library constructed from a *desA*-Δ mutant of *Synechocystis* sp. PCC 6803 (Sakamoto *et al.*, 1994a) was screened by cross-hybridization with the 0.75-kbp probe derived from the *desC* gene of *A. variabilis* (Fig. 1*A*). The nucleotide sequence of a region that hybridized with the probe was determined for one of the positive clones. An open reading frame of 954 nucleotides was found (Fig. 1*B*) that encodes a polypeptide of 318 amino acid residues. The extent of the similarity between the deduced amino acid sequence of this open reading frame (Fig. 2) and that of the open reading frame of the *desC* gene of *A. variabilis* was 62%. Therefore, the gene in *Synechocystis* sp. PCC 6803 that encodes an open reading frame of 318 amino acids is a homologue of *desC* in *A. variabilis*. Thus, we tentatively iden-

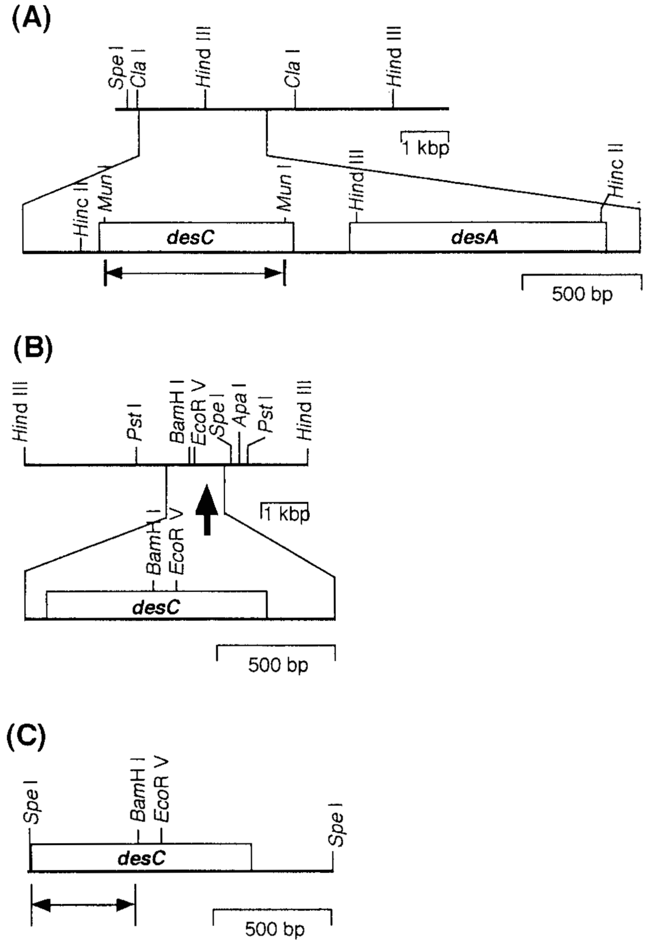

FIG. 1. **Restriction maps for the cloned DNA fragments.** *A*, the restriction map of the DNA fragment of 7 kbp that contained the *desA* and *desC* genes of *A. variabilis* (Sakamoto *et al.*, 1994b). The probe of 0.75 kbp (position 315 to position 1,070 in the nucleotide sequence) for the screening of the genomic library of *Synechocystis* sp. PCC 6803 was prepared with *Mun*I digestion, and it was labeled with [α-^{32}P]dCTP using a random primer labeling kit (Takara, Kyoto, Japan). This probe is indicated by a double-headed arrow. *B*, the restriction map of the 6-kbp insert of pBluescript/H6 that contained the *desC* gene of *Synechocystis* sp. PCC 6803. The region that hybridized with the 0.75-kbp probe is indicated by an *arrow*. *C*, the restriction map of the 1.1-kbp insert of pET3a/*desC*(S). The region of 0.5 kbp that was amplified by polymerase chain reaction is indicated by a *double-headed arrow*.

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6803         1  MLNPLNIEYLYLSKLFDNSLIVFNKRQLFRFFVRFFFMTAALPNDSKPKLTPAWTVIF   58
Anabaena     1                                           MTIATST.PQIN.VNTL   17
Rat         19  ITEPPSGNLQNGREKMKKVPLYLEEDIRPEMREDIHDPSYQDEEGPP...EYV.RN.I   76
Mouse       16  TTTITAPPSGNEREKVKTVPLHLEEDIRPEMKEDIHDP.YQDEEGPP...EYV.RN.I   73
OLE1        61  .VSVEFDKKGNEK.SNLDR.LEKDNQEKEEAKTKIHISEQPWTLNNWHQHLNWLNMVL  118


6803            FFTSIHLVALLAFLPQFFSWKAVGMAFLLYVITGGIGITLGFHRCISHRSFNVPKWLEYI  118
Anabaena        ..LGL.IG..F..I.SN...A..GV.L...W....L........LVT....QT......F   77
Rat             LMALL.VG..YGITLIPS.KVYTLLWGIF.YLISAL...A.A..LW...TYKARLP.RIF  136
Mouse           LMVLL..GG.YGIILVPSCKLYTALFGIF.YM.SAL...A.A..LW...TYKARLP.RIF  133
OLE1            VCGMPMIGWYF.LSGKVPLHLN.FLFSVF.YAV..VS..A.Y..LW....YSAHWP.RLF  178
                                             *       ** * **   **       *


6803            FVICGTLACQGGVFEWVGLHRMHHKFSDTTPDPHDSNKGFWWSHIGWMMFEIPAKADIPR  178
Anabaena        L.L.........PI....T..I..LH...D.................LIYHS.SH..VP.  137
Rat             LI.AN.M.F.ND.Y..ARD..A.....E.HA...N.RR..FF..V..LLVRKHPAVKEKG  196
Mouse           LI.AN.M.F.ND.Y..ARD..A.....E.HA...N.RR..FF..V..LLVRKHPAVKEKG  193
OLE1            YA.F.CASVE.SAKW.GHS..I..RYT..LR..Y.ARR.L.Y..M...LLKPNP.YKARA  238
                               *   ** **    *  **     •   ** **
                                   _____


6803            ---YTKDIQDDKFYQFCQNNLILIQVALGLILFALGGWPFV--------IWGIFVRLVFV  227
Anabaena        ---F....AE.PV...L.KYF.F..I....L.LY....S..--------VW.V.F.I.W.  186
Rat             GKLDMS.LKAE.LVM.QRRYYKPGLLLMCF..PT.VP.YCWGETFLHSLFVST.L.YTL.  256
Mouse           GKLDMS.LKAE.LVM.QRRYYKPGLLLMCF..PT.VP.YCWGETFVNSLFVST.L.YTL.  253
OLE1            ---DIT.MT..WTIR.QHRHY..LMLLTAFVIPT.ICGY.FND-YMGGL.YAG.I.VFVI  294
                      *        *                  *                  * *


6803            FHFTWFVNSATHKFGYVSHESNDYSRNCWWVALLTFGEGWHNNHHAYQYSARHGLQWWEV  287
Anabaena        Y.C..L.........RTYDAG.R.T......V.V...........F........E....  246
Rat             LNA..L....A.LY..RPYDK.IQ..ENIL.S.GSV...F..Y...FP.DYSASEYR.HI  316
Mouse           LNA..L....A.LY..RPYDK.IQ..ENIL.S.GAV...F..Y..TFPFDYSASEYR.HI  313
OLE1            QQA.FCI..MA.YI.TQPFDDRRTP.DN.IT.IV.....Y..F..EFPTDY.NAIK.YQY  354
                   *   **  *  *                     *** ** **
                                                        _____


6803            DLTWMTIKFLSLLGLAKDIKLPPETAMANKA                               318
Anabaena        ......VQL.QI....TNV..ADKKQ                                    272
Rat             NF.TFF.DCMAA....Y.R.KVSKA.VLARIKRTGDGSHKSS                    358
Mouse           NF.TFF.DCMAA....Y.R.KVSKATVLARIKRTGDGSHKSS                    355
OLE1            .P.KVI.YLT..V...Y.L.KFSQN.IEEALIQQEQKKINKKKAKINWGPVLTDLPMWDK  414
                  *          ***   •
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FIG. 2. **The amino acid sequences deduced from the *desC* genes of *Synechocystis* sp. PCC 6803 and *A. variabilis*, compared with the amino acid sequences of stearoyl-CoA desaturases from the rat (Thiede *et al.*, 1986), the mouse (Ntambi *et al.*, 1988), and *S. cerevisiae* (*OLE1*; Stukey *et al.*, 1990).** The conserved amino acid residues are indicated by *asterisks*. The amino acid sequence motif -H-*X*-*X*-H-H- is indicated by a *bar*. The gaps that were introduced to maximize similarity are indicated by *dashes*.

tified the gene as the *desC* gene of *Synechocystis* sp. PCC 6803.

Expression of the Two desC Genes in E. coli—The *desC* genes of *A. variabilis* and *Synechocystis* sp. PCC 6803 were subcloned separately into pET3a. Successful introduction of each gene for a desaturase into *E. coli* BL21(DE3)pLysS and the directed expression of the genes under control of the T7 bacteriophage promoter were detected by monitoring changes in the fatty acid composition of membrane lipids. Before induction of the Δ9 acyl-lipid desaturase by IPTG, there were no significant differences between the fatty acid compositions of membrane lipids from *E. coli* cells that had been transformed with pET3a, pET3a/*desC*(A) or pET3a/*desC*(S) (Table I). The cells contained saturated and monounsaturated fatty acids, such as 12:0, 14:0, 16:0, 16:1(9), 18:0, and 18:1(11). In addition, they contained a relatively high level of 18:0 (*i.e.* 10–20% of the total fatty acids), because the *E. coli* cells had been supplied with stearic acid to increase the basal level of 18:0, which was usually less than 1% when the cells were cultured without stearic acid (data not shown). Upon incubation with IPTG for 1 h to allow expression of the introduced *desC* genes, the level of 18:0 decreased and that of 18:1(9) increased significantly in the cells that had been transformed with pET3a/*desC*(A) or with pET3a/*desC*(S). An increase in the level of 18:1(9) was observed in all lipid classes, PE, PG, and CL. It should be noted that levels of 16:0 and 16:1(9) and 18:1(11) did not change in these cells. By contrast, the cells that had been transformed with pET3a did not show any increase in the level of 18:1(9) during the incubation with IPTG. These results confirm that the *desC* genes of *A. variabilis* and *Synechocystis* sp. PCC 6803 encode Δ9 acyl-lipid desaturases which are specific to stearic acid.

Table II shows distributions of fatty acids at the *sn*-positions of glycerol moiety in PE and PG after the transformed *E. coli*

TABLE I
Changes in the fatty acid composition of individual lipid classes upon expression of the desC genes in E. coli

Values are averages (±S.D.) of results obtained from three independent cultures of each transformant. t, trace (less than 0.5%).

Lipid class	Fatty acid					
	14:0	16:0	16:1(9)	18:0	18:1(9)	18:1(11)
			mol %			
Before induction						
pET3a						
PE (78%)	2	31 ± 2	25 ± 1	14 ± 2	t	25 ± 1
PG (21%)	1	27 ± 2	17 ± 1	16 ± 1	1	36 ± 1
CL (1%)	1	32 ± 1	14 ± 1	19 ± 2	2	32 ± 2
pET3a/*desC*(A)						
PE (79%)	3 ± 1	35 ± 1	24 ± 1	11 ± 1	1	24 ± 1
PG (20%)	1	31 ± 1	16 ± 1	13 ± 1	1	36 ± 1
CL (1%)	1	31 ± 2	12 ± 1	15 ± 1	3 ± 1	38 ± 2
pET3a/*desC*(S)						
PE (80%)	3 ± 1	34 ± 1	24 ± 1	10 ± 1	2	26 ± 1
PG (19%)	1	31 ± 1	17 ± 1	10 ± 1	3 ± 1	36 ± 1
CL (1%)	0	30 ± 1	11 ± 1	10 ± 2	5 ± 1	39 ± 1
After induction by IPTG for 1 h						
pET3a						
PE (82%)	4 ± 1	36 ± 3	24 ± 1	11 ± 2	t	23 ± 3
PG (17%)	1	30 ± 2	15 ± 1	14 ± 1	1	39 ± 1
CL (1%)	1	36 ± 1	12 ± 1	16 ± 2	1	33 ± 2
pET3a/*desC*(A)						
PE (78%)	2	34 ± 1	22 ± 1	12 ± 1	3 ± 1	25 ± 1
PG (20%)	1	30 ± 1	16 ± 1	13 ± 1	5 ± 1	34 ± 1
CL (2%)	1	28 ± 2	13 ± 2	14 ± 2	6 ± 1	38 ± 2
pET3a/*desC*(S)						
PE (74%)	3 ± 1	33 ± 1	24 ± 1	9 ± 1	6 ± 1	24 ± 1
PG (21%)	1	30 ± 1	19 ± 1	8 ± 1	10 ± 1	31 ± 1
CL (5%)	1	27 ± 1	18 ± 1	8 ± 1	10 ± 1	36 ± 1

TABLE II
Positional distribution of fatty acids in individual lipid classes isolated from E. coli cells that had been transformed with the desC genes and induced by IPTG

Values are averages of results obtained from three independent cultures of each transformant. The deviation of the values was less than 2%. t, trace (less than 0.5%).

Lipid class (position)	Fatty acid					
	14:0	16:0	16:1(9)	18:0	18:1(9)	18:1(11)
			mol %			
pET3a (control)						
PE						
(C-1)	1	68	6	16	t	5
(C-2)	3	4	42	6	1	41
PG						
(C-1)	1	51	12	16	t	20
(C-2)	1	8	18	11	3	58
pET3a/*desC*(A)						
PE						
(C-1)	1	63	3	16	5	12
(C-2)	3	4	41	8	1	38
PG						
(C-1)	1	55	13	7	8	15
(C-2)	1	5	19	19	2	53
pET3a/*desC*(S)						
PE						
(C-1)	1	61	7	9	11	11
(C-2)	3	5	41	9	1	37
PG						
(C-1)	1	51	16	1	18	14
(C-2)	1	7	22	18	2	48

cells had been incubated with IPTG for 1 h. Notably, 18:1(9) was specifically esterified to the C-1 positions of PE and PG in cells that had been transformed with pET3a/*desC*(A) and pET3a/*desC*(S). These results indicate that the Δ9 acyl-lipid desaturases of *A. variabilis* and *Synechocystis* sp. PCC 6803 desaturate 18:0, but not 16:0, at the C-1 position of phospholipids and that these enzymes do not discriminate among polar head groups.

DISCUSSION

In the present study, we isolated *desC* genes of *A. variabilis* and *Synechocystis* sp. PCC 6803 that encode Δ9 acyl-lipid desaturases, and we characterized the genes using an expression system under the control of T7 RNA polymerase in *E. coli*. In *E. coli* transformants that expressed the *desC* gene of *A. variabilis* or *Synechocystis* sp. PCC 6803, 18:1(9) accumulated at the expense of 18:0 at the C-1 position of the glycerol moiety of PE and PG (Table II). It is unlikely that the product of each *desC* gene desaturated 18:0 to 18:1(9) in the ACP-bound form, with the resultant 18:1(9) being subsequently and selectively esterified to the C-1 position of PE and PG. It has been demonstrated that 18:1(9)-ACP is a poor substrate for the glycerol-3-phosphate acyltransferase of *E. coli* (Rock *et al.*, 1981). Therefore, it is very likely that 18:0 was desaturated in the lipid-bound form in the transformed cells of *E. coli*, just as it is during in the desaturation reactions in cyanobacteria.

The electron donor for desaturation reactions in cyanobacterial cells is ferredoxin (Wada *et al.*, 1993). *E. coli* cells contain ferredoxin (Knoell and Knappe, 1974). Therefore, it is very likely that the cyanobacterial desaturases expressed in *E. coli* cells accepted electrons from the host's ferredoxin.

As previously mentioned, it has been established that fatty acids in cyanobacteria are desaturated in the glycerolipid-bound form (Sato and Murata, 1982; Sato *et al.*, 1986). In *Synechocystis* sp. PCC 6803, which is a member of Group 4 of cyanobacteria with respect to the way in which fatty acids are desaturated (Murata *et al.*, 1992), only 18:0 esterified to the C-1 position of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, PG, and sulfoquinorosyldiacylglycerol is desaturated, whereas 16:0 esterified to either the C-1 or the C-2 position of glycerolipids is not desaturated (Wada and Murata, 1990). These features of desaturation reactions in cyanobacteria are consistent with those of the desaturation reactions in *E. coli* cells that had been transformed with the *desC* genes. Therefore, we conclude that the Δ9 acyl-lipid desaturase of *Synechocystis* sp. PCC6803 is 1) specific to stearic acid, 2) specific to the C-1 position of the glycerol moiety, and 3) nonspecific with respect to the polar head group.

Fig. 2 compares the deduced amino acid sequences of the Δ9 acyl-lipid desaturases of *A. variabilis* and *Synechocystis* sp. PCC 6803 with those of Δ9 stearoyl-CoA desaturases of the rat (Thiede *et al.*, 1986), the mouse (Ntambi *et al.* 1988) and *S. cerevisiae* (Stukey *et al.*, 1990). The extent of sequence similarity between the Δ9 acyl-lipid desaturase of *Synechocystis* sp. PCC 6803 and the Δ9 stearoyl-CoA desaturases of the rat, the mouse, and *S. cerevisiae* was found to be 25, 24, and 25%, respectively. The Δ9 stearoyl-ACP desaturases from castor bean (Shanklin and Somerville, 1991; Knutzon *et al.*, 1991), safflower (Thompson *et al.*, 1991), cucumber (Shanklin *et al.*, 1991), spinach (Nishida *et al.*, 1992), rape seed (Knutzon *et al.*, 1992), and the Δ4 palmitoyl-ACP desaturase from coriander (Cahoon *et al.*, 1992, Cahoon and Ohlrogge, 1994) are very different from the Δ9 acyl-lipid desaturases in terms of their amino acid sequences and hydropathy profiles (data not shown).

The Δ9 acyl-lipid desaturase of *Synechocystis* sp. PCC 6803 is not very similar to the other acyl-lipid desaturases from the same cyanobacterium in terms of amino acid sequence. The extent of similarity is 15, 13, and 13% for the Δ6, Δ12, and ω3 desaturases, respectively. However, the hydropathy profiles of the four desaturases of *Synechocystis* sp. PCC 6803 are rather similar. Each has two major hydrophobic regions, a property that is consistent with the finding that these desaturases are membrane-bound proteins (Wada *et al.*, 1993).

Histidine residues are well conserved between Δ9 acyl-lipid desaturases and Δ9 18:0-CoA desaturases (Fig. 2). Ten of fifteen histidine residues in the Δ9 acyl-lipid desaturases are conserved in the Δ9 18:0-CoA desaturases. In particular, two clusters of histidine residues, namely, -H-*X*-*X*-H-H- can be found in both types of Δ9 desaturase, as indicated in Fig. 2. The two histidine clusters, which are conserved in the Δ9 acyl-lipid desaturases, are also found in other desaturases of *Synechocystis* sp. PCC 6803 (data not shown). Since the two histidine clusters are located in the hydrophilic regions, they may be involved in binding of iron atoms, as occurs in the Δ9 18:0-ACP desaturase of castor bean (Fox *et al.*, 1993). The similarities among the partial sequences of the desaturases suggest that a common mechanism may exist for the introduction of a double bond into a hydrocarbon chain. It is likely that the two histidine clusters play an essential role in catalyzing the desaturation of fatty acids. They may, for example, transport electrons from ferredoxin to the site of desaturation of fatty acids.

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