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Cloning of ω3 desaturase from cyanobacteria and its use in altering the degree of membrane-lipid unsaturation

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Abstract

Cyanobacteria respond to a decrease in temperature by desaturating fatty acids of membrane lipids to compensate for the decrease in membrane fluidity. Among various desaturation reactions in cyanobacteria, the desaturation of the ω3 position of fatty acids is the most sensitive to the change in temperature. In the present study, we isolated a gene, designated *desB*, for the ω3 desaturase from the cyanobacterium, *Synechocystis* sp. PCC 6803. The *desB* gene encodes a protein a 359 amino-acid residues with molecular mass of 41.9 kDa. The *desB* gene is transcribed as a monocistronic operon that produced a single transcript of 1.4 kb. The level of the *desB* transcript in cells grown at 22 °C was 10 times higher than that in cells grown at 34 °C. In order to manipulate the fatty-acid unsaturation of membrane lipids, the *desB* gene in *Synechocystis* sp. PCC 6803 was mutated by insertion of a kanamycin-resistance gene cartridge. The resultant mutant was unable to desaturate fatty acids at the ω3 position. The *desA* gene, which encodes the Δ12 desaturase of *Synechocystis* sp. PCC 6803, and the *desB* gene were introduced into *Synechococcus* sp. PCC 7942. Whilst the parent cyanobacterium can only desaturate membrane lipids at the Δ9 position of fatty acids, the resultant transformant was able to desaturate fatty acids of membrane lipids at the Δ9, Δ12 and ω3 positions. These results confirm the function of the *desB* gene and demonstrate that it is possible to genetically manipulate the fatty-acid unsaturation of membrane lipids in cyanobacteria.

Introduction

Most cyanobacterial strains contain high levels of polyunsaturated fatty acids, as do higher plants [19]. However, the biosynthetic pathways of polyunsaturated fatty acids in cyanobacteria and higher plants differ considerably. All desaturation reactions of fatty acids in cyanobacteria take place

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number D13780 (*desB* gene).

after the fatty acids have become bound to glycerolipids [28, 29]. In higher plants, the desaturation of stearic acid to oleic acid occurs when the fatty acid is in the ACP-bound form [18, 37], and all other desaturation reactions take place after the fatty acids have become bound to glycerolipids [7, 11]. In higher plants, the enzyme that catalyzes the desaturation of stearoyl-ACP is a water-soluble protein which is localized in the stroma of chloroplasts. By contrast, the desaturases, which catalyze the desaturation of fatty acids bound to lipids, are membrane-bound [33]. These membrane-bound desaturases in higher plants and cyanobacteria are called 'acyl-lipid desaturases'.

Ferredoxin is involved in the desaturation of fatty acids as an electron donor in chloroplasts of higher plants [30] and also in cells of cyanobacteria [45]. By contrast, cytochrome b_5 is the electron donor of fatty-acid desaturation in endoplasmic reticula of higher plants [12, 34].

In the cyanobacterium *Synechocystis* sp. PCC 6803, polyunsaturated fatty acids are synthesized by the sequential introduction of double bonds into C_{18} fatty acids, which have been esterified to the C-1 position of glycerolipids. This is catalyzed by four distinct desaturases, each of which has positional specificity [41, 42]. A study of the substrate specificity of the desaturases of *Synechocystis* sp. PCC 6803, which involved feeding cells with an odd-numbered aliphatic acid [9], demonstrated that each of the desaturases introduces a double bond at the Δ6, Δ9, Δ12 or ω3 position of fatty acids respectively. The gene for Δ12 desaturase, *desA* [43, 46], and the gene for Δ6 desaturase [24] have been cloned from *Synechocystis* sp. PCC 6803. However, genes for the other two desaturases have not been isolated.

The unicellular cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 can be transformed with exogenously added DNA [8]. This property has enabled us to examine the biological role of the unsaturation of fatty acids of membrane lipids by analyzing the effects of manipulation of the *desA* gene. Disruption of the *desA* gene in *Synechocystis* sp. PCC 6803 results in elimination of the desaturation of fatty acids at the Δ12 position and renders the cyanobacterium sensitive to low temperature [6, 44]. Introduction of the *desA* gene into *Synechococcus* sp. PCC 7942 allowed this strain to desaturate fatty acids at the Δ12 position and increased its tolerance to low temperature [43, 47].

In the present report we describe the isolation of the *desB* gene of *Synechocystis* sp. PCC 6803, which is responsible for acyl-lipid desaturation at the ω3 position of fatty acids, by cross-hybridization with a probe derived from the *desA* gene. By genetic manipulation using the *desB* gene, we have been able to create cyanobacterial strains with different levels of unsaturation of the fatty acids in membrane lipids. These should be useful in future physical characterization of the membranes.

Materials and methods

Organisms and culture conditions

Synechocystis sp. PCC 6803 and *Synechococcus* sp. PCC 7942 were grown photoautotrophically at 34 °C or 22 °C in BG-11 medium supplemented with 20 mM HEPES–NaOH (pH 7.5) as described previously [41]. Illumination was provided by incandescent lamps at 70 $\mu E\ m^{-2}\ s^{-1}$. Mutants of *Synechocystis* sp. PCC 6803 and transformants of *Synechococcus* sp. PCC 7942 were selected and maintained on BG-11 medium [35] supplemented with kanamycin at 25 μg/ml and streptomycin at 10 μg/ml for both liquid culture and growth on plates.

Construction of Synechocystis *sp. PCC 6803 mutant* desA-Δ

We created a mutant of *Synechocystis* sp. PCC 6803, termed *desA*-Δ, in which all copies of the *desA* gene in the genome were replaced by a Km^r cartridge [3] as follows. A *desA* probe of 1.2 kb (position 1 to position 1233 in the nucleotide sequence deposited in the EMBL databank with the accession number X53508) was prepared by

digestion of 'Bluescript/1.5-kbp' [43] with *Eco* RI and *Spe* I, and subsequently labelled with [α-^{32}P]dCTP using a random primer labelling kit (Takara, Kyoto, Japan). Screening of a λEMBL3 genomic library of wild-type *Synechocystis* sp. PCC 6803 provided by Dr J.G.K. Williams (E.I. DuPont de Nemours and Company) with the *desA* probe resulted in the isolation of a clone with a 14 kb insert. An *Eco* RI fragment of 3.0 kb, which corresponds to the region upstream of the *desA* gene, was obtained from the λEMBL3 clone. This fragment was subcloned into the *Eco* RI site of the plasmid, pBluescript II KS(+) (Stratagene, La Jolla, CA), and the resultant plasmid designated pBluescript/E3. The *Spe* I fragment of 1.0 kb, which corresponds to the region downstream of the *desA* gene, was obtained from the same λEMBL3 clone. This fragment was subcloned into the *Spe* I site of pBluescript II KS(+), and the resultant plasmid designated pBluescript/S1. The Km^r (kanamycin resistance gene) cartridge of 1.2 kb was prepared from pUC4 KIXX (Pharmacia, Uppsala, Sweden) by digestion with *Sma* I. This cartridge was inserted into the *Eco* RV site of pBluescript/E3 and the resultant plasmid designated pBluescript/E3/Km. The 4.2 kb fragment containing the 3.0 kb fragment and the 1.2 kb Km^r cartridge was prepared by digesting pBluescript/E3/Km^r with *Sma* I and *Sal* I and generation of blunt ends by T4 DNA polymerase. The 4.2 kb fragment was inserted into the *Hinc* II site of pBluescript/S1. The resultant plasmid, containing the construct illustrated as in Fig. 1A(2), was designated pBluescript/E3/Km^r/S1.

Wild-type cells of *Synechocystis* sp. PCC 6803 were transformed with pBluescript/E3/Km/S1 by the method of Golden *et al.* [5]. The resultant transformant was designated *desA*-Δ. The genomic DNA of the *desA*-Δ mutant was analyzed by DNA/DNA blot hybridization with the Km^r probe of 1.2 kb and the *desA* probe of 1.2 kb under highly stringent conditions in Rapid Hybridization Buffer (Amersham, Buckinghamshire, UK) using the manufacturers recommended protocol.

Screening of the genomic library of the desA-Δ *mutant*

Genomic DNA of the *desA*-Δ mutant, extracted as described by Williams [48], was partially digested with *Sau*3A I, and integrated into the *Bam* HI site of the phage vector *λDASH* II (Stratagene). The resultant genomic library was screened by plaque hybridization essentially as described by Ausubel *et al.* [2]. Approximately 3.5×10^3 plaques were transferred onto nylon membranes (GeneScreen Plus; DuPont/NEN Research Products, Boston, MA), and hybridized with a *desA* probe of 0.25 kb in 0.09 M sodium citrate buffer (pH 7.0) containing 0.9 M NaCl, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 10% dextran sulfate, 0.1% SDS and 100 μg/ml salmon sperm DNA for 42 h at 50 °C. The probe (positions 416 to 661 in the nucleotide sequence deposited in the EMBL databank with the accession number X53508) was prepared by digestion of 'Bluescript/1.5-kbp' with *Eco*O109 I and labelling with [$\alpha = {}^{32}$P]dCTP using a random primer labelling kit (Takara). After hybridization, the membranes were washed at 50 °C with 0.015 M sodium citrate buffer (pH 7.0) that contained 0.15 M NaCl and 0.5% SDS, and then exposed to X-ray films (WIF50; Konica, Tokyo, Japan).

Nucleotide and deduced amino-acid sequences

The *Eco* RI DNA fragment of 6.6 kb from one of the positive clones was subcloned into the *Eco* RI site of pBluescript II KS(+). The resultant plasmid was designated pBluescript/6.6-kbp. The nucleotide sequence of the 1.7 kb, which contained the region that hybridized with the *desA* probe of 0.25 kb, was determined by the dideoxychain termination method [27] using the 7-deaza dGTP DNA sequencing kit (Takara), the *Bca*-BEST dideoxy sequencing kit (Takara) and the 7-deaza dGTP sequenase kit (U.S. Biochemical Corporation, Cleveland, OH). The entire sequences of both strands in this region were determined. The alignment of amino-acid sequences

was performed using the molecular evolutionary analysis system for DNA and amino-acid sequences, ODEN, at National Institute of Genetics (Mishima, Japan).

Northern blot analysis

Isolation of the RNA from *Synechocystis* sp. PCC 6803 and northern blot analysis were performed as described previously [17]. A desB probe of 830 bp for the hybridization was prepared by amplifying the appropriate region of the genomic DNA of *Synechocystis* sp. PCC 6803 by the polymerase chain reaction with the following primers: the forward primer, 5′-TTGCAGTCTTAAAG-TTTTGACG-3′ (positions from −246 to −225 of the sequence shown in Fig. 3) and the reversed primer, 5′-TCCGGACGGAATAGGG-GACTGC-3′ (positions from 582 to 560 of the sequence shown in Fig. 3). The amplified DNA fragment was identified by Southern blot analysis, which was performed under the same conditions as northern blot hybridization. The DNA fragment was labelled with [α-^{32}P]dCTP using a nick translation kit (Takara). The size of the *desB* transcript was determined using RNA molecular weight markers I (Boehringer, Mannheim, Germany).

Disruption of the desB *gene in* Synechocystis *sp. PCC 6803*

A disrupted *desB* gene, designated *desB*::Km^r, was constructed by inserting the Km^r cartridge (described above) into the *Sac* I site of the coding region of the *desB* gene, as illustrated in Fig. 1B, as follows. The plasmid containing the *desB* gene in an insert of 3.5 kbp was prepared by digestion of pBluescript/6.6-kbp with *Bam* HI and subsequent self-ligation. The resultant plasmid was partially digested with *Sac* I, blunt-ended by T4 polymerase and ligated with the Km^r cartridge. The wild-type strain of *synechocystis* sp. PCC 6803 was transformed with the plasmid DNA which included the disrupted *desB* gene as described by Golden *et al.* [5]. The resultant transformant was designated WT/*desB*::Km^r. Genomic DNA of WT/*desB*:Km^r was analyzed by the polymerase chain reaction using primers derived from the *desB* gene (the forward primer, 5′-TTACCATTTACCCTCCAA-3′ and the reverse primer, 5′-TTCAATTAGGATCA-ATTAAG-3′; indicated by arrows in Figs. 1B and 3). Products of polymerase chain reaction were analyzed by agarose gel electrophoresis.

Integration of the desA *gene into the chromosome of* Synechococcus *sp. PCC 7942*

We generated a transformant, designated 7942/*desA*, in which the *desA* gene was integrated in the chromosome of *Synechococcus* sp. PCC 7942, by integrating the *desA* gene and the Km^r cartridge into the *Eco* RI site of the coding region of the *cmpA* gene, as illustrated in Fig. 1C. The *cmpA* gene in the genome of *Synechococcus* sp. PCC 7942 [22] was amplified by the polymerase chain reaction using the primers for *cmpA* (the forward primer, 5′-ATGAACGAATTTCAACCAGT-3′ and the reverse primer, 5′-TGCCCGTTTAA-TCTTCAAGC-3′; indicated by small arrows in Fig. 1C). The resultant product was subcloned into the *Sma* I site of modified pBluescript II KS(+), in which the *Eco* RI site had been deleted from the multicloning site by digestion with *Xho* I and *Pst* I and the product blunt-ended by T4 DNA polymerase followed by subsequent self-ligation. The resultant plasmid was designated pBluescript/*cmpA*. A DNA fragment of 1.2 kb containing the *desA* gene was prepared by digesting 'Bluescript/1.5-kbp' [43] with *Eco* RI and *Sma* I and blunt-ended by T4 DNA polymerase. This fragment was inserted into the *Sma* I site of pBluescript/S1, which contained the 1.0 kb fragment downstream of the *desA* gene. The resultant plasmid was designated pBluescript/*desA*/S1. pBluescript/E3/Km^r, described above, was digested with *Sma* I and *Sal* I to produce a 4.2 kb fragment that contained the 3.0 kb fragment and the 1.2 kb Km^r cartridge. This fragment was blunt-ended by T4 DNA polymerase and inserted

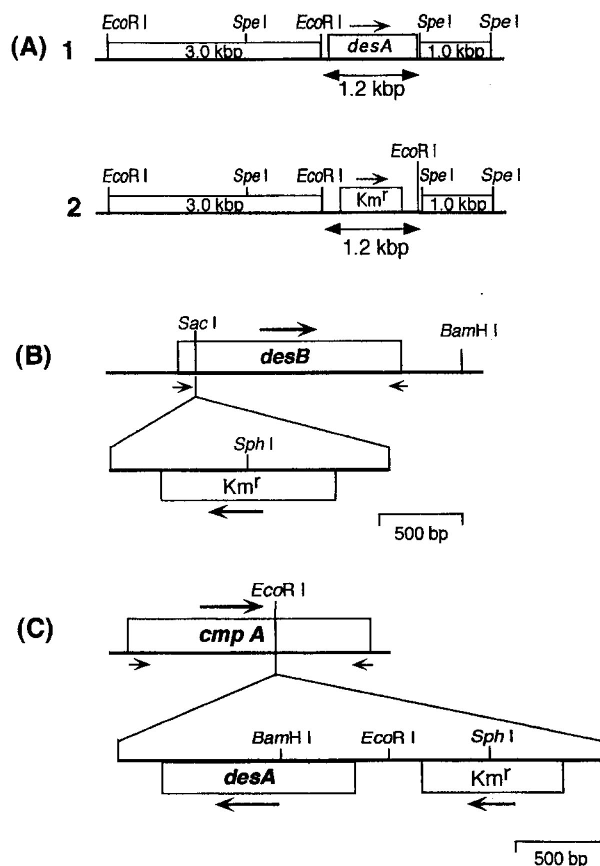

Fig. 1. Constructs of DNA fragments for the mutation of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942. (A) Replacement of the *desA* gene by the Km^r cartridge. The 3.0 kb fragment upstream and the 1.0 kb fragment downstream of the *desA* gene were combined with the Km^r cartridge of 1.2 kb from pUC4 KIXX as illustrated in Ausubel *et al.* [2]. This construct, as an insert of 5.2 kb between the *Hinc* II and *Spe* I sites of pBluescript II KS(+), was used to transform the wild-type strain of *Synechocystis* sp. PCC 6803 to yield the *desA*-Δ mutant by homologous recombination. (B) The insertional disruption of the *desB* gene with the Km^r cartridge. The Km^r cartridge of 1.2 kb was inserted into the *Sac* I site in the open-reading frame of the *desB* gene, which was cloned between the *Eco* RI and *Bam* HI sites of pBluescript II KS(+). The resultant construct was used to transform the wild-type strain of *Synechocystis* sp. PCC 6803 to generate the WT/*desB*::Km^r mutant by homologous recombination. Small arrows indicate the primers for the polymerase chain reaction. (C) The insertion of the *desA* gene and the Km^r cartridge into the *cmpA* gene of *Synechococcus* sp. PCC 7942. The *desA* gene and the Km^r cartridge were inserted into the *Eco* RI site of the *cmpA* gene [22] that had been cloned in pBluescript II KS(+). The resultant construct was used to transform the wild-type strain of *Synechococcus* sp. PCC 7942 to generate the 7942/*desA* transformant. Small arrows indicate the primers for the polymerase chain reaction.

into the *Xho* I site of pBluescript/*desA*/S1, blunt-ended by T4 DNA polymerase. The resultant plasmid was designated pBluescript/E3/Km^r/*desA*/S1. The pBluescript/E3/Km^r/*desA*/S1 was digested with *Sma* I and *Xho* I to prepare the 2.4 kb fragment that contained the *desA* gene and the Km^r cartridge. After blunt-ending by T4 DNA polymerase, the 2.4 kbp fragment was inserted into the *Eco* RI site of pBluescript/*cmpA*, blunt-ended by T4 DNA polymerase. The resultant plasmid, which contained the construct illustrated in Fig. 1C, was designated pBluescript/*cmpA*::*desA*.

The wild-type strain of *Synechococcus* sp. PCC 7942 was transformed with the pBluescript/*cmpA*::*desA* by the method of Williams and Szalay [49]. The genomic DNA from the resultant transformant, 7942/*desA*, was analyzed by the polymerase chain reaction using the *cmpA* primers described above. Products of polymerase chain reaction were analyzed by agarose gel electrophoresis.

Introduction of the desB *gene into* Synechococcus *sp. PCC 7942*

The 6.6 kb *Eco* RI fragment of pBluescript/6.6-kbp, containing the *desB* gene, was subcloned into the *Eco* RI site of pUC303, a shuttle vector between *Escherichia coli* and *Synechococcus* sp. PCC 7942 [15]. The resultant plasmid, designated pUC303/6.6-kbp, was used to transform wild-type and 7942/*desA* cells. The transformation was accomplished essentially as described by Williams and Szalay [49]. Streptomycin-resistant transformants were selected on BG-11 plates that contained 10 μg/ml streptomycin. The transformants of wild-type and 7942/*desA* cells were designated transformant 7942/*desB* and double-transformant 7942/*desA*/*desB*, respectively.

Analysis of fatty acids

Cells collected by centrifugation were lyophilized and subjected to methanolysis with 5% HCl

(w/w) in methanol at 85 °C for 2.5 h. The resultant methyl esters were extracted by *n*-hexane and analyzed by gas-liquid chromatography as described previously [41]. Methyl esters of fatty acids were identified with a gas-liquid chromatograph and a gas chromatograph-mass spectrometer. The positions of double bonds were determined by a gas chromatograph-mass spectrometer [41].

Results

Evaluation of the desA-Δ *mutant*

The *desA*-Δ mutant of *Synechocystis* sp. PCC 6803 was produced as described in Materials and methods. Since *Synechocystis* sp. PCC 6803 contains six to eight copies of its chromosomal DNA per cell [48], it was important to confirm that the *desA* gene had been replaced by the Km^r cartridge in the *desA*-Δ mutant in all copies of the chromosomal DNA. The genomic DNA from wild-type and *desA*-Δ cells was analyzed by DNA/DNA blot hybridization with the probes derived from the *desA* gene and the Km^r cartridge.

The DNA from the wild-type cells hybridized with the *desA* probe of 1.2 kb giving a band of 1.1 kb. No band was detected with the Km^r probe (Fig. 2A). The DNA from *desA*-Δ cells hybridized with the Km^r probe giving a band of 1.3 kb, but it did not hybridize with the *desA* probe (Fig. 2A). These results indicate that the *desA* gene had been replaced by the Km^r cartridge in all copies of the chromosomal DNA in the *desA*-Δ mutant.

In the *desA*-Δ mutant, levels of 18:1(9) and 18:2(6,9) (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) were significantly increased whereas 18:3(6,9,12) and 18:2(9,12) had disappeared (data not shown). The fatty-acid composition of the *desA*-Δ mutant was similar to that of our previously described Fad12 and WT/*desA*::Km^r mutants [41, 43], which are defective in fatty-acid desaturation at the Δ12 position. This result indicates that the *desA*-Δ mutant had lost the ability to desaturate fatty acids at the Δ12 position.

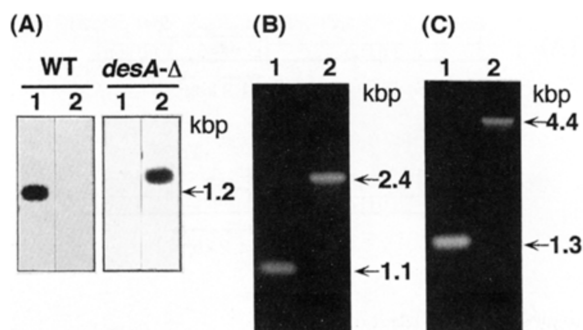

Fig. 2. Evaluation of the replacement of genes in the chromosomes of *desA*-Δ, WT/*desB*::Km^r and 7942/*desA* cells. (A) Analysis by DNA/DNA hybridization to demonstrate the complete replacement of the *desA* gene by the Km^r cartridge in *desA*-Δ cells. The genomic DNA from wild-type and *desA*-Δ cells of *Synechocystis* sp. PCC 6803 was digested with *Eco* RI and *Spe* I and blotted onto nylon membranes. The 1.2 kb *desA* probe (lane 1) and the 1.2 kb Km^r probe (lane 2) were used for hybridization. (B) Analysis by the polymerase chain reaction to demonstrate the complete replacement of the native *desB* gene by the disrupted *desB* gene in WT/*desB*::Km^r cells. Genomic DNA from wild-type (lane 1) and WT/*desB*::Km^r (lane 2) cells was used as template for the polymerase chain reaction with the primers indicated by small arrows in Fig. 1B. (C) Analysis by the polymerase chain reaction to demonstrate the integration of the *desA* gene and the Km^r cartridge into the *cmpA* gene in 7942/*desA* cells. The genomic DNA isolated from wild-type (lane 1) and 7942/*desA* (lane 2) cells of *Synechococcus* sp. PCC 7942 was used as template for the polymerase chain reaction with the primers indicated by small arrows in Fig. 1C.

Cloning of the desB *gene from* Synechocystis *sp. PCC 6803*

The genomic DNA library of the *desA*-Δ mutant, constructed in the phage vector *λDASH* II, was screened with the 0.25 kb *desA* probe. About 260 putative positive clones were obtained from 3.5×10^3 recombinants, and 26 clones were arbitrarily selected among these positive clones. The restriction maps and the size of the DNA fragments that hybridized with the screening probe allowed us to divide them into seven groups. We determined a partial nucleotide sequence of the insert of a representative from each of the seven groups, and found that one of the seven groups had sequence similarity to the *desA* gene. A clone of this group gave a fragment of 6.6 kb upon

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-375                                                TTCGAGTGATTCCCCATCGCATCGA
-360 ATTTTGGCAAGGCCGCCCTAGTCGTCTCCATGACCGTTTGCAATTTAATTTGCTTGATGGTCAATGGCACAG
-288 ACAGAGGTTGGCGCCGTAGAAGTTAAACTAGCCCCCATTCCATTGCAGTCTTAAAGTTTTGACGTTTTAGTC
                                                                       -35
-216 CATAAAGTTGATCAGAGTTGCGTATTTATAAACATTTGAACTTTTTATCGTCCTTTCTCTACACAAATTTGA
                 -10
-144 ATCAAGTATTCTCCTTAGTGTGTTTTCTGTCTTAGTCAAAATTGGTCTTACTGCCGAATTTTCATCTTCCAA

 -72 CGGCAGCCTTCTTTGAAGATTTAGGATAGAATCATAGGATTGTTTTGCCGTCATAGCCCCTAAGATAAATAC
                                                                   S.D.

   1 GTGCGTCTAGAAATTTCATCGCCTCAAACAAAGCTTCCTTACCCCAAAACTGAAGAATTACCATTTACCCTC
   1 ValArgLeuGluIleSerSerProGlnThrLysLeuProTyrProLysThrGluGluLeuProPheThrLeu

  73 CAAGAGCTCAGAAACGCTATTCCAGCGGATTGTTTTGAGCCATCGGTAGTCCGGTCCTTGGGCTACTTTTTT
  25 GlnGluLeuArgAsnAlaIleProAlaAspCysPheGluProSerValValArgSerLeuGlyTyrPhePhe

 145 TTGGATGTTGGTTTAATTGCCGGGTTTTATGCTCTAGCGGCCTACCTTGATTCCTGGTTCTTCTATCCGATT
  49 LeuAspValGlyLeuIleAlaGlyPheTyrAlaLeuAlaAlaTyrLeuAspSerTrpPhePheTyrProIle

 217 TTTTGGTTAATTCAGGGAACCCTATTCTGGTCCCTGTTTGTGGTGGGCCATGATTGTGGCCATGGCTCCTTT
  73 PheTrpLeuIleGlnGlyThrLeuPheTrpSerLeuPheValValGlyHisAspCysGlyHisGlySerPhe

 289 TCCAAATCCAAAACCCTTAATAATTGGATTGGTCATCTCAGCCACACGCCAATTTTGGTGCCTTACCATGGC
  97 SerLysSerLysThrLeuAsnAsnTrpIleGlyHisLeuSerHisThrProIleLeuValProTyrHisGly

 361 TGGCGTATTAGTCATCGTACTCACCATGCCAACACGGGCAATATCGACACCGACGAAAGTTGGTATCCAGTG
 121 TrpArgIleSerHisArgThrHisHisAlaAsnThrGlyAsnIleAspThrAspGluSerTrpTyrProVal

 433 TCGGAGCAAAAATATAACCAAATGGCCTGGTATGAAAAACTTCTACGTTTTTACTTGCCTCTGATCGCCTAC
 145 SerGluGlnLysTyrAsnGlnMetAlaTrpTyrGluLysLeuLeuArgPheTyrLeuProLeuIleAlaTyr

 505 CCCATTTATCTATTTCGGCGATCGCCAAACCGGCAAGGCTCCCATTTCATGCCCGGCAGTCCCCTATTCCGT
 169 ProIleTyrLeuPheArgArgSerProAsnArgGlnGlySerHisPheMetProGlySerProLeuPheArg

 577 CCCGGAGAAAAAGCAGCTGTTCTCACCAGCACCTTTGCCCTTGCAGCCTTTGTCGGCTTCCTTGGCTTTTTA
 193 ProGlyGluLysAlaAlaValLeuThrSerThrPheAlaLeuAlaAlaPheValGlyPheLeuGlyPheLeu

 649 ACTTGGCAATTTGGCTGGCTATTTTTGCTGAAATTTTATGTTGCCCCCTACCTCGTGTTTGTGGTGTGGTTA
 217 ThrTrpGlnPheGlyTrpLeuPheLeuLeuLysPheTyrValAlaProTyrLeuValPheValValTrpLeu

 721 GATTTGGTCACATTTTTACATCACACTGAAGACAATATCCCTTGGTATCGTGGTGATGACTGGTATTTTCTC
 241 AspLeuValThrPheLeuHisHisThrGluAspAsnIleProTrpTyrArgGlyAspAspTrpTyrPheLeu

 793 AAAGGTGCCCTCTCCACCATTGATCGGGATTACGGCTTCATTAACCCCATTCACCATGACATTGGCACCCAC
 265 LysGlyAlaLeuSerThrIleAspArgAspTyrGlyPheIleAsnProIleHisHisAspIleGlyThrHis

 865 GTCGCCCACCATATTTTCTCGAATATGCCCCACTACAAGTTACGCCGGGCGACTGAAGCCATCAAGCCCATT
 289 ValAlaHisHisIlePheSerAsnMetProHisTyrLysLeuArgArgAlaThrGluAlaIleLysProIle

 937 TTAGGGGAATATTATCGATATTCTGACGAGCCAATTTGGCAAGCTTTTTTTAAGTCCTACTGGGCTTGCCAT
 313 LeuGlyGluTyrTyrArgTyrSerAspGluProIleTrpGlnAlaPhePheLysSerTyrTrpAlaCysHis

1009 TTTGTTCCTAATCAAGGTTCAGGGGTCTATTACCAATCCCCATCCAATGGTGGATATCAAAAGAAACCTTAA
 337 PheValProAsnGlnGlySerGlyValTyrTyrGlnSerProSerAsnGlyGlyTyrGlnLysLysPro***

1081 TTGATCCTAATTGAATCAAACCAACATCGGGAGGGAAGGCAATATTAGATGGTATTACTCCCTTCCGATCGC
1153 CTTATGAGAAGTATGGAAGAAGTTAAACAATCACACTCCAAAAGATCAGCATTAAACCAACAACGGTGCTGT
1225 GGTGAGGCAGAAGATTGAAAAAGGATTGTTTACCTAACTGTTGACTGAGAAAAATCGACCAAAGTACCCCGG
1297 CCAAAAGCAGCATACCTTGCAAAAAGGCGATCGCCGCTGGATCC
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Fig. 3. The nucleotide sequence of the *desB* gene of *Synechocystis* sp. PCC 6803 and the deduced amino acid sequence. The sequences are numbered with 1 representing the initiation codon of the *desB* gene. The putative promoter elements, −35, −10, and the Shine-Dalgarno Sequence (S.D.) are indicated in the 5′-upstream region of the open-reading frame of the *desB* gene. Sequences corresponding to the primers, which were used for analysis of the disruptant mutants, are indicated by arrows.

digestion with *Eco* RI, and this fragment was subcloned into the *Eco* RI site of pBluescript II KS(+). The nucleotide sequence of 1725 bp in the hybridizable region of the fragment was determined, and an open-reading frame was found. As will be confirmed below, this open-reading frame corresponds to the gene for the ω3 desaturase. This gene was designated *desB*.

Sequence of the desB *gene*

The nucleotide sequence of the *desB* gene and the deduced amino-acid sequence are shown in Fig. 3. An open-reading frame of 1077 nucleotides was found, which corresponds to a polypeptide of 359 amino acid residues with an approximate molecular mass of 41.9 kDa. The Shine-Dalgarno sequence in the 5′-upstream region of the open-reading frame of the *desB* gene resembles the corresponding sequence of *E. coli* [25]. Although the site of the transcriptional initiation was not experimentally determined, putative sequences at the −35 and −10 regions were identified (Fig. 3).

Temperature-dependent expression of the desB *gene*

In order to investigate the temperature-dependent expression of the *desB* gene, total RNA, prepared from wild-type cells of *Synechocystis* sp. PCC 6803 that had been incubated at designated temperatures for 18 h, was subjected to northern blot hybridization (Fig. 4). Only one hybridizable band with a mobility that corresponded to 1.4 kb

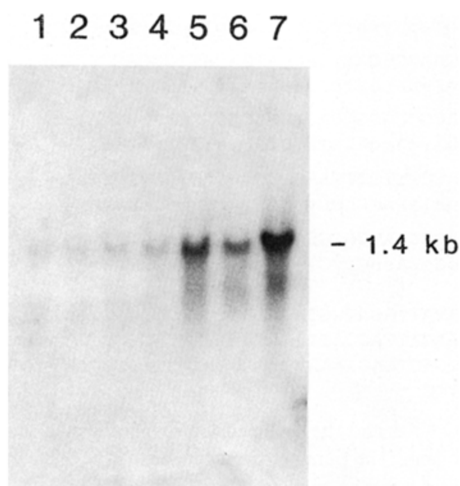

Fig. 4. The effect of incubation at various temperatures on the level of the *desB* transcript in *Synechocystis* sp. PCC 6803, analyzed by northern blot hybridization. Cells were grown isothermally for 3 days at 34 °C (lane 1) and were incubated for 18 h at 32 °C (lane 2), 30 °C (lane 3), 28 °C (lane 4), 26 °C (lane 5), 24 °C (lane 6), and 22 °C (lane 7).

appeared at the various temperatures. This result suggests that the *desB* gene was transcribed as a monocistronic operon producing a single transcript. A transcript of the *desB* gene was barely detected in cells grown at 34 °C. A low level of the transcript was observed in cells incubated at 28 °C (Fig. 4). However, a remarkable accumulation of the transcript was detected at 26 °C, and the level of the transcript increased to reach maximum at 22 °C (Fig. 4). These observations coincide with the result of analysis of fatty-acid composition, namely, that 18:3(9,12,15) and 18:4(6,9,12,15) are not found in cells grown at 34 °C [42]. The level of the transcript of the *desB* gene increased about 10-fold with the decrease in temperature from 34 °C to 22 °C, suggesting that the desaturation at the $\omega 3$ position at low temperatures is regulated by the expression of the *desB* gene.

Disruption of the desB *gene in* Synechocystis *sp. PCC 6803*

In order to confirm that the *desB* gene in WT/*desB*::Km^r had been disrupted by insertion of the Km^r cartridge in all of the copies of the chromosomal DNA, the genomic DNA from wild-type and WT/*desB*::Km^r cells was analyzed by the polymerase chain reaction. A DNA fragment of 1.1 kb, which originated from the native *desB* gene, was amplified by the polymerase chain reaction with the DNA from wild-type cells (Fig. 2B, lane 1). A DNA fragment of 2.4 kb, which was expected to include the *desB* gene that had been disrupted by insertion of the Km^r cartridge, was amplified by the polymerase chain reaction with the DNA from WT/*desB*::Km^r cells (Fig. 2B, lane 2). These results confirm that all copies of the *desB* gene in WT/*desB*::Km^r were disrupted by the insertion of the Km^r cartridge.

Fatty-acid compositions of the total lipids from wild-type and WT/*desB*::Km^r cells are compared in Table 1. Wild-type cells grown at 34 °C contained 18:1(9), 18:2(9,12) and 18:3(6,9,12) as major fatty acids. The fatty-acid composition of WT/*desB*::Km^r cells grown at 34 °C was the same

Table 1. Changes in the fatty-acid composition of total lipids upon disruption of the *desB* gene in *Synechocystis* sp. PCC 6803.

Strain	Fatty acid								
	16:0	16:1 (9)	18:0	18:1 (11)	18:1 (9)	18:2 (9,12)	18:3 (6,9,12)	18:3 (9,12,15)	18:4 (6,9,12,15)
Grown at 34 °C									
Wild type	60 ± 1	4 ± 1	1	1	11 ± 1	13 ± 1	10 ± 1	0	0
WT/*desB*: :Km^r	62 ± 2	5 ± 1	1	1	9 ± 1	12 ± 1	10 ± 1	0	0
Grown at 22 °C									
Wild type	55 ± 1	3 ± 1	1	1	4 ± 1	10 ± 1	16 ± 2	6 ± 1	4 ± 1
WT/*desB*: :Km^r	54 ± 2	5 ± 1	1	1	4 ± 1	16 ± 2	19 ± 2	0	0

Values were obtained from three independent cultures.

as that of the wild-type cells. When wild-type cells were grown at 22 °C, 18:3(9,12,15) and 18:4(6,9,12,15) appeared in addition to the fatty acids present at 34 °C. However, these two fatty acids did not emerge in WT/*desB*::Km^r cells grown at 22 °C. This result indicates that the WT/*desB*::Km^r strain had lost the ability to introduce a double bond into the $\omega 3$ position of fatty acids and, moreover, that the *desB* gene is responsible for the desaturation of fatty acids at the $\omega 3$ position.

Transformation of Synechococcus *sp. PCC 7942*

Wild-type cells of *Synechococcus* sp. PCC 7942 were transformed with the *desA* gene and the Km^r cartridge, which had been integrated into the *cmpA* gene as in Fig. 1C. Genomic DNA from wild-type and 7942/*desA* cells was analyzed by the polymerase chain reaction in order to confirm the integration of this construction into the *cmpA* gene. A DNA fragment of 1.3 kb, which had originated from the native *cmpA* gene, was amplified from DNA of wild-type cells (Fig. 2C, lane 1). By contrast, a DNA fragment of 4.4 kbp, which was expected from the *cmpA* gene modified by integration of the *desA* gene and the Km^r cartridge, was amplified from the DNA of 7942/*desA* cells (Fig. 2C, lane 2). A DNA fragment of 1.3 kb, corresponding to the native *cmpA* gene, was not detected (Fig. 2C, lane 2). These findings confirm that both the *desA* gene and the Km^r cartridge had been inserted into the *cmpA* gene in all copies of the chromosomal DNA of the 7942/*desA* strain.

Wild-type and 7942/*desA* cells were transformed with pUC303/6.6-kbp that contained the *desB* gene from *Synechocystis* sp. PCC 6803, as described in Materials and methods. The transformants of wild-type and 7942/*desA* cells, designated 7942/*desB* and 7942/*desA*/*desB*, respectively, were isolated and examined.

Fatty-acid compositions of total lipids from wild-type, 7942/*desA*, 7942/*desB* and 7942/*desA*/*desB* cells are shown in Table 2. The wild-type cells grown at 34 °C contained saturated and monounsaturated fatty acids such as 16:0, 16:1(9), 18:1(9) and 18:1(11). In the 7942/*desA* cells, 16:2 (9,12) and 18:2(9,12) increased, and 16:1(9) and 18:1(9) decreased. By contrast, the fatty-acid composition of 7942/*desB* cells was the same as that of wild-type cells. 7942/*desA*/*desB* cells contained 18:3(9,12,15) in addition to 16:2(9,12) and 18:2(9,12). Incubation of these cells at 22 °C for 18 h increased the degree of desaturation of 16:1(9) and 18:1(9) in 7942/*desA* cells, and of 18:2(9,12) in 7942/*desA*/*desB* cells. These results indicate that the 7942/*desA*/*desB* strain acquired the ability to introduce a double bond at the $\omega 3$ position of fatty acids upon transformation with both *desA* and *desB* genes. The cells transformed with the *desB* gene alone, 7942/*desB*, were unable to desaturate fatty acids at the $\omega 3$ position.

Table 2. Changes in the fatty-acid composition (mol %) of total lipids upon transformation of *Synechococcus* sp. PCC 7942 with the *desA* and *desB* genes.

Strain	Fatty acid									
	16:0	16:1 (9)	16:2 (9,12)	16:3[1] (?)	18:0	18:1 (11)	18:1 (9)	18:2 (9,12)	18:2[1] (?)	18:3 (9,12,15)
Grown at 34 °C										
Wild type (7942)	54 ± 3	34 ± 2	0	0	4 ± 1	3 ± 1	5 ± 1	0	0	0
Transformants										
7942/*desA*	54 ± 2	25 ± 1	9 ± 1	0	3 ± 1	3 ± 1	t	4 ± 1	2 ± 1	0
7942/*desB*	53 ± 2	36 ± 2	0	0	2 ± 1	6 ± 1	3 ± 1	0	0	0
7942/*desA*/*desB*	56 ± 2	24 ± 1	8 ± 1	1	4 ± 1	3 ± 1	t	3 ± 1	2 ± 1	1
Grown at 34 °C, then incubated at 22 °C for 18 h										
Wild type (7942)	46 ± 1	40 ± 2	0	0	3 ± 1	6 ± 1	4 ± 1	0	0	0
Transformants										
7942/*desA*	45 ± 2	18 ± 2	14 ± 2	0	4 ± 1	5 ± 1	t	7 ± 1	8 ± 2	0
7942/*desB*	50 ± 2	35 ± 2	0	0	4 ± 1	6 ± 1	6 ± 1	0	0	0
7942/*desA*/*desB*	47 ± 2	18 ± 2	11 ± 1	4 ± 1	4 ± 1	5 ± 1	t	3 ± 1	4 ± 1	5 ± 1

[1] 16:3(?) and 18:2(?) are hexadecatrienoic and octadecadienoic acids, respectively, but the sites of their double bonds were not determined.

t, trace amount (less than 0.5%).

Values were obtained from three independent experiments.

Discussion

In the present study, we have isolated the gene for the ω3 desaturase from *Synechocystis* sp. PCC 6803. Using a technique for the insertional inactivation of genes, we have produced a mutant with a disrupted *desB* gene that was unable to desaturate fatty acids at the ω3 position. By introduction of genes for desaturase into *Synechococcus* sp. PCC 7942, we have produced transformants capable of desaturating fatty acids at the ω3 position. These results indicate that the electron-donating system in *Synechococcus* sp. PCC 7942 is an effective donor for the ω3 desaturase of *Synechocystis* sp. PCC 6803.

Figure 5 compares amino-acid sequences deduced from the *desB* and *desA* genes and the *fadD* cDNAs that encode plastid ω3 desaturases of higher plants, *Arabidopsis thaliana* [10, 50], *Glycine max* [50] and *Brassica napus* [50]. The extents of similarity in terms of the amino acid sequence between the cyanobacterial ω3 desaturase and the plastid ω3 desaturases of *G. max*, *A. thaliana* and *B. napus* are 49%, 49%, and 50%, respectively. They are lower than those between the plastid ω3 desaturases, which range from 66% to 94%. The similarity between the *desB* gene and the *desA* gene is small, namely, 28%. Although the similarity of amino-acid sequence between the cyanobacterial and higher-plant ω3 desaturases is not significant, there are four conserved domains (Fig. 5, indicated by boxes). The first domain of the ω3 desaturases is similar to the corresponding domain of the Δ12 desaturases of

Fig. 5. Alignment of amino acid sequences deduced from the *desB* gene, the *fadD* cDNAs which encode putative plastid ω3 desaturases of *Glycine max*, *Arabidopsis thaliana* and *Brassica napus* [50], and the *desA* gene [43] that encodes the Δ12 desaturase of *Synechocystis* sp. PCC 6803. Identical amino acids are indicated by dots. The conserved amino acids in all sequences are indicated by asterisks. The domains conserved in the ω3 desaturases are boxed. The domains conserved among the Δ12 desaturases of cyanobacteria [26] are indicated by underlines. The conserved nine amino acid sequence motif is indicated by a bar with arrowheads. The histidine residues conserved in the *desA* and the *desB* genes of *Synechocystis* sp. PCC 6803 are indicated by arrowheads.

```
desB
Glycine           MATWYHQKCGLKPLAPVIPRPRTGAALSSTSRVEFLDTNKVVAGPKFQPLRCNLR   55
Arabidopsis                MANLVLSECGIRPLPRIYTTPRSNFLSNNNKFRPSLSSSSYKTSSSP   58
Brassica                                                             FKFRQ    5
desA

desB                                   VRLEI--SSPQTKLPYPKTEELPFTLQELRNAIPA   33
Glycine      ERNWGLKVSAPLRVASIEEEQKSVDLTNGTNGVEHEKLPEFDPGAPP..N.ADI.A...K  115
Arabidopsis  LSFGLNSRDGFTRNWALNVSTPLTTPIF.ESPLEEDN.QRFDPGAPP..N.ADI.A...K  107
Brassica     SPSSPRFRLNSRNWALNVTTPLTVDSSSSPPIEEEPKTQRFDPGAPP..N.ADI.A...K   65
desA                                   MTAT.PPLT.TVTPSN.DRPIADLK..DIIKTL.K   35
                                                                *       *

desB         DCFEPSVVRSLGYFFLDVGLIAGFYALAAYLDSWFFYPIFWLI|QGTLFWSLFVVGHDCGH   93
Glycine      H.WVKDPW..MS.VVR..IAVF.LA.A....NN.LVW.LY.AA|...M..A...L......  175
Arabidopsis  H.WVKNPWK..S.VVR..AIVFALA.G....NN.IVW.LY..A|...M..A...L......  167
Brassica     H.WVKNPWK.MS.VVRELAIVFALA.G....NN.LVW.LY.IA|...M..A...-......  124
desA         E...KKASKAWASVLITL.A..VG.LGII..-P.YCL.IT.IW T..ALTGA.........   94
              *                                **   *    *   *    **      ** ******

desB         GSFS|KSKTLNNWIGHLSHTP|ILVPYHGWRISHRTHH|ANTGNIDTDESWYPVSE---QKYN  150
Glycine      ....|NNSK..SVV...L.SS|................|QHH.HAEN....H.LP.KLFRSLD  235
Arabidopsis  ....|NDPK..SVV...L.SS|................|Q.H.HVEN....H.M..KIYNTLD  227
Brassica     ....|NDPR..SVV...L.SS|................|Q.H.HVEN....H.MEK-IYKSLD  183
desA         R..A .KRWV.DLV..IAFA. LIY.F.S..LL.DH.. LH.NK.EV.NAWD.W.V---EAFQ  151
              **       *   **        * * **   *  **           *   * *

desB         QMAWYEKLLRFYLPL--IAYPIYLFRRSPNRQGSHFMPGSPLFRPGEKAAVLTST-FALA  207
Glycine      TVT---RM...TA.FPLL.F.V...S...GKT....D.S.D..V.N.RKD.I...ACWA.  292
Arabidopsis  KPT---RFF..T...VML...F..WA...GKK...YH.D.D..L.K.RKD.....ACWT.  284
Brassica     KPT---RFF..T...VML...F..WA...GKK...YH.D.D..L.K.RND..-..ACWT.  239
desA         ASP---AIV.LFYRA--.RG.FWWTGSIFHWSLM..KLSNFAQ.DRN.VKL-SIAVVF.F  205
                      *           *                *

desB         AFVGFLGFLTWQFGWLFLLKFYVAPYLVFVV|WLDLVTFLHH--TEDNIPWYRG|DDWYFLK  265
Glycine      MLGLLV.-.GFVM.PIQ...L.GV..VI..M|......Y...HGH..KL.....|KE.SY.R  351
Arabidopsis  MAALLVC-.NFTI.PIQM..L.GI..WIN.M|...F..Y...HGH..KL.....|KE.SY.R  343
Brassica     MA.LLVC-.NFVM.PMQM..L..I..WIN.M|...F..Y...HGH..KL...-.|KE.SY.R  297
desA         .AIA.PA-.IITT.VWGFV..WLM.W..YHF .MSTF.IV..--.IPE.RFRPA A..SAAE  262
                  *    *      *     *         *     *  **              *

desB         GALSTI|DR-DYG-FINPIHHDIGTHVAHH|IFSNMPHYKLRRATEAIKPILGEYYR-YSDE  322
Glycine      .G.T.L|..-...-W..N.........I..|L.PQI...H.VE....A..VF.K...EPKKS  409
Arabidopsis  .G.T.L|..-...-L..N.........I..|L.PQI...H.VE....A..V..K...EPDKS  401
Brassica     .G.T.L|..-...-L..N.........I..|L.PQI...H.VE....A..V..-...EPDKS  394
desA         AQ.NGT VHC..PRWVEVLC...NV.IP.. LSVAI.S.N..L.HGSL.ENW.-PFL-.ERT  320
               *       **         ***  *  **      * * *  *    *   *

desB         -P-----IWQAFFKSYWACHFVPNQGSGVYYQSPSNGGYQKKP     359
Glycine      AAPLPFHLIGEIIR.FKTD...SDT.DV....TD.KINGSS.L--E  453
Arabidopsis  -GPLPLHLLEILA..IKED.Y.SDE.EV...KADP.LYGEV.VRAD  446
Brassica     -GPLPLHLLGILA..IKED...SDE.DV...EADP.LYGEI.VTAE  399
desA         -F-----N..LMQQISGQ..LYDPE------HGYRTF.SL..V     351
                                *                      *
```

cyanobacteria (Fig. 5 indicated by underlining [26]). By contrast, the amino-acid sequences of the other conserved domains of the ω3 desaturases differ from those of the corresponding domains of the Δ12 desaturase of *Synechocystis* sp. PCC 6803. It is likely that the first domain plays an essential role in the desaturation of fatty acids bound to membrane lipids, while the other domains are related to the introduction of a double bond into the ω3 position of fatty acids.

The *desB* gene was compared with the *fad3* cDNAs of higher plants which encode the cytoplasmic ω3 desaturases (not shown as a figure). The extents of similarity between the deduced amino-acid sequence from the cyanobacterial ω3 desaturase and the deduced sequences from the cytoplasmic ω3 desaturases of *Vigna radiata* [51], *G. max* [50], *A. thaliana* [10, 50], the *B. napus* [1, 50] are 47%, 48%, 46% and 45%, respectively.

Figure 6 compares the hydropathic characteristics of the products of the *desB* and *desA* genes (Fig. 6). The predicted product of the *desB* gene contains two hydrophobic regions and, therefore, is likely to be a membrane-bound protein. The

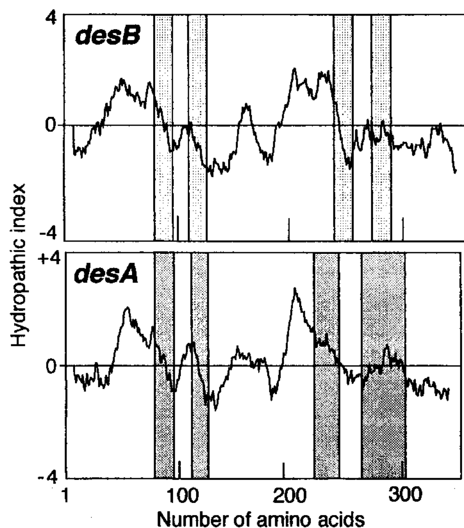

Fig. 6. Hydropathy profile of the product of the *desB* gene compared with that of the *desA* gene. The four domains conserved in the ω3 desaturases and the four domains conserved in the Δ12 desaturases of cyanobacteria [26] are indicated by shadowing. The hydropathic index was calculated according to the algorithm of Kyte and Doolittle [16] for a window size of 19 amino acid residues.

four conserved domains among the ω3 desaturases are located in the hydrophilic regions or at the border between the hydrophobic and the hydrophilic regions (Fig. 6, indicated by shadowing). The hydropathy profiles of the plastid and cytoplasmic ω3 desaturases of higher plants are similar to that of the product of the *desB* gene (data not shown).

The similarity between the deduced amino-acid sequences of a cyanobacterial Δ6 desaturase [24] and the cyanobacterial ω3 desaturase is very small, namely, 16%. Little similarity is seen between the cyanobacterial ω3 desaturase and the Δ9 desaturases from a number of different organisms when compared at the amino acid level, i.e., the Δ9 stearoyl-CoA [21, 36, 38] and the Δ9 stearoyl-(acyl-carrier-protein) desaturases [13, 14, 20, 31, 32, 39].

Histidine residues are well conserved in the ω3 and Δ12 desaturases of *Synechocystis* sp. PCC 6803 (Fig. 5, indicated by arrowheads). Thirteen of 15 histidine residues are located within the four domains that are conserved among the ω3 desaturases. These histidine residues are also included in the conserved domains among the Δ12 desaturases of cyanobacteria (Fig. 5 indicated by underlining [26]). It is notable that histidine is the amino-acid residue conserved between the Δ9 stearoyl-CoA desaturases from rat [38] and yeast [36]. Although the role of the histidine residues in the desaturation of fatty acids has not been clarified, we can assume that these residues contribute to the catalytic function of the desaturases.

The level of the *desB* transcript was about 10 times higher in cells incubated at low temperatures, such as 22 °C, 24 °C, 26 °C, than in cells grown at 34 °C. This result is compatible with the result of the analysis of fatty-acid composition, namely, 18:3(9,12,15) and 18:4(6,9,12,15) accumulated in wild-type cells of *Synechocystis* sp. PCC 6803 grown at low temperatures [42]. The low-temperature-induced accumulation of the transcript of the *desB* gene is also compatible with our recent finding that the transcription of the *desA* gene is accelerated by a decrease in temperature [17], as well as by hydrogenation of lipids of the plasma membrane [40].

Recently, a sequence motif conserved in low-temperature-inducible promoters of *E. coli*, CCAAT, was identified by comparing the 5′-upstream nucleotide sequences of low-temperature-inducible genes [23]. Since the motif, CCAAT, was not found in the 5′-non-coding region of the *desB* gene, the mechanisms for the induction of the *desB* gene at low temperature may be different from that of the low-temperature-inducible genes of *E. coli*.

In 7942/*desA* cells, that is, *Synechococcus* sp. PCC 7942 cells transformed with the *desA* gene, the *desA* gene and the Km^r cartridge were inserted into the *cmpA* gene. It had been shown [22] that there are no significant differences between wild-type and mutant cells with a disrupted *cmpA* gene in terms of growth rates under either low or high CO_2 conditions. Therefore, it is unlikely that the disruption of the *cmpA* gene itself affects the desaturation of fatty acids.

The introduction of the double bond at the $\omega3$ position occurred only when *Synechococcus* sp. PCC 7942 cells were transformed with both the *desA* and the *desB* genes. By contrast, the desaturation of fatty acids at the $\omega3$ position did not occur when the *desB* gene alone was introduced into the wild-type cells. These results suggest that the $\omega3$ desaturase is active when fatty acids contain a double bond at the $\Delta12$ position. This observation is compatible with our previous observation [41] that the Fad12 mutant of *Synechocystis* sp. PCC 6803, which is defective in the $\Delta12$ desaturase, cannot introduce a double bond at the $\omega3$ position.

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