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cemA homologue essential to CO_2 transport in the cyanobacterium *Synechocystis* PCC6803

(*cotA*/mutant/HCO_3^- transport/chloroplast envelope)

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ABSTRACT We have isolated mutants of *Synechocystis* PCC6803 that grew very slowly in a low-sodium medium, which is unfavorable for HCO_3^- transport, and examined two of these mutants (SC1 and SC2) for their ability to take up CO_2 and HCO_3^- in the light. The CO_2 transport activity of SC1 and SC2 was much lower than that of the wild type (WT), whereas there was no difference between the mutants and the WT in their activity of HCO_3^- transport. A clone containing a 3.9-kilobase-pair insert DNA that transforms both mutants to the WT phenotype was isolated from a genomic library of WT *Synechocystis*. Sequencing of the insert DNA in the region of mutations in SC1 and SC2 revealed an open reading frame (designated *cotA*), which showed significant amino-acid sequence homology to *cemA* encoding a protein found in the inner envelope membrane of chloroplasts. The *cotA* gene is present in a single copy and was not cotranscribed with any other gene(s). *cotA* encodes a protein of 247 amino acids containing four transmembrane domains. There was substitution of a single base in SC1 and two bases in SC2 in their *cotA* genes. A possible role of the *cotA* gene product in CO_2 transport is discussed.

The CO_2-concentrating mechanism in cyanobacteria involves an active transport of inorganic carbon (C_i; CO_2 and HCO_3^-) driven by photosystem I cyclic electron flow (1–5). Both CO_2 and HCO_3^- are the species transported into the cells by this mechanism, and HCO_3^- appears to be the only species delivered to the interior of the cells (5, 6). The cyanobacterial C_i-transport system has a high affinity to CO_2 but has a low affinity to HCO_3^- (4, 6–8) (e.g., $K_{1/2}$ values of 1.6 and 141 μM for CO_2 uptake and HCO_3^- uptake, respectively, for air-grown cells of *Synechococcus*; ref. 8). Bicarbonate uptake requires the presence of Na^+ in the millimolar range, but a very low concentration of Na^+ (in the micromolar range) is large enough to saturate the response of CO_2 uptake to Na^+ (4, 9, 10). These differences between CO_2 uptake and HCO_3^- uptake in their kinetic parameters and Na^+ requirement encouraged us to isolate mutants defective in CO_2 transport. It was assumed that such mutants may not or may very slowly grow under low concentrations of Na^+ and/or HCO_3^- in the medium, the conditions unfavorable for HCO_3^- transport.

Identification of gene(s) involved in C_i transport is one of the most important steps in elucidating the CO_2-concentrating mechanism. This study aims at isolation and characterization of the gene(s) involved in CO_2 transport. For this purpose, we isolated mutants of *Synechocystis* PCC6803 whose growth rate is much lower than that of the wild type (WT) with air levels of CO_2 in a low-Na^+ medium. Preliminary physiological data on one of these mutants (SC; SC1 in this paper) have been shown elsewhere (11). We describe in this paper that two mutants, SC1 and SC2, are defective in CO_2 transport and that both mutants have mutations in the gene, designated *cotA*, which encodes a protein homologous to CemA identified in the inner envelope membrane of pea chloroplasts (12). The *cemA* gene and its homologues have been found in chloroplast genomes of a number of plants (13–17). These genes are assumed to encode b-type heme proteins (17), but their function remains unknown. Physiological and molecular analyses of the SC1 and SC2 mutants indicated that the *cemA* homologue in *Synechocystis* (*cotA*) encodes a protein required for CO_2 transport.

MATERIALS AND METHODS

Isolation of Mutants. Cells of *Synechocystis* PCC6803 were grown at 30°C in BG11 medium (18) supplemented with 20 mM Hepes–KOH buffer (KOH added to pH 8.0) during aeration with 3% vol/vol CO_2 in air. Low-sodium medium was prepared by adding NaCl (final concentration, 100 μM) to the BG11 medium in which all of the sodium salts were replaced by potassium salts. Continuous illumination was with tungsten lamps providing 120 μmol of photosynthetically active radiation/m^2·s. Cells in a logarithmic phase of growth were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (40 μg/ml) for 20 min in the light, washed twice, and grown with 3% CO_2/97% air in BG11 medium. Cells were then grown for 2 days with air in the presence of ampicillin (100 μg/ml) in the low-Na^+ (100 μM) BG11 medium. The activity of HCO_3^- uptake in *Synechocystis* PCC6803 at this Na^+ concentration was much lower than at 15 mM Na^+ (see curves a and e in Fig. 2*C*), whereas there was no difference in the activity of CO_2 uptake at these two different concentrations of Na^+ (data not shown). This confirms the validity of the strategy for the mutant isolation. After the ampicillin treatment, cells were plated on BG11/agar plates and incubated under 3% CO_2/97% air in the light until colonies appeared. Each colony was transferred to duplicate plates—a normal BG11 plate containing 17 mM Na^+ and the low Na^+ BG11 plate. The normal BG11 plate was incubated under 3% CO_2 and the low-Na^+ plate under air. Seven mutants were rescued as colonies that grew slowly or not at all on the low-Na^+ plate. Two of these mutants, SC1 and SC2, were subjected to physiological and molecular analyses.

Gas Exchange Measurements. Air-grown cells of the WT and mutant strains were suspended in 25 ml of 20 mM Hepes–KOH buffer (pH 7.0) containing 100 μM NaCl and placed in a reaction vessel at a chlorophyll (Chl) level of 5 μg/ml. CO_2 exchange of the cell suspension was measured at 30°C by using an open gas-analyses system (19). Air containing 340 μl of CO_2 per liter was led to the reaction vessel at a flow

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Abbreviations: WT, wild type; C_i, inorganic carbon; CA, carbonic anhydrase; ORF, open reading frame; Chl, chlorophyll.

The sequence reported in this paper is deposited in the GenBank data base (accession no. D63838).

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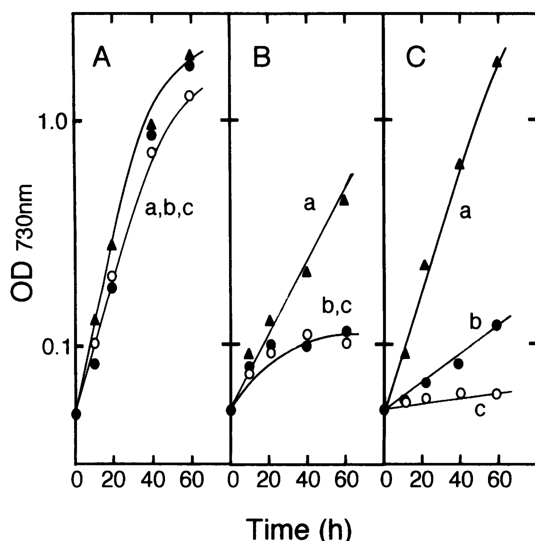

FIG. 1. Growth curves of WT (curve a), SC1 (curve b), and SC2 (curve c) cells in BG11 medium (pH 7.0) during aeration with air (340 ppm CO_2) (*A*), with 60 ppm CO_2 in CO_2-free air (*B*), and with air in a low-Na^+ BG11 medium (100 μM; pH 8.0) (*C*).

rate of 1.0 liter/min, and the CO_2 concentration in the exchanged gas was analyzed with an infrared gas analyzer.

Silicone-Oil Filtering Centrifugation. Time courses of uptake of $^{14}CO_2$ and $H^{14}CO_3^-$ into the intracellular C_i pool of the WT and mutant cells were measured by the silicone oil-filtering centrifugation method (6). Cells were harvested by centrifugation and resuspended in 20 mM Hepes–KOH buffer (pH 8.0) containing 15 mM (or 100 μM) NaCl at a Chl concentration of 20 μg/ml. C_i uptake was initiated by the addition of $^{14}CO_2$ or $H^{14}CO_3^-$ in the light and was terminated by centrifugation.

Other Measurements. Growth curves were determined from the increase in the OD at 730 nm by using a Hitachi (Tokyo) recording spectrophotometer (model 2000–10). Pigments in the cells were extracted by methanol, and Chl concentration in the extract was determined (20).

Cloning and Transformation. A clone that complements the mutants was isolated from a partial genomic library of WT *Synechocystis* constructed by ligating 3- to 4-kbp DNA fragments from a *Bam*HI digest into the pKY184 vector (21). A DNA fragment containing the contiguous region of the complementing clone was isolated from a λDASH library of WT *Synechocystis* by plaque hybridization with a 1.1-kbp *Bam*HI/*Spe* I fragment as a probe (see Fig. 3). Complementation test was performed by the method of transformation reported by Dzelzkalns and Bogorad (22). After application of DNA (≈50 ng) on BG11 plates (17 mM Na^+, pH 7) containing mutant cells in a top agar, the plates were incubated in the light under 60 ppm of CO_2. Transformants were visible in 7 days.

Analysis of Mutant DNA. Fragments of the *cotA* gene of SC1 and SC2 were amplified by the PCR method (23) with the genomic DNA of the mutants as the templates and were cloned into the TA vector (Invitrogen) for sequencing. The oligonucleotides used as the primers were synthesized according to the sequence shown in Fig. 4 (sequences from base 989 to 1005 for SC1 and from base 630 to 646 for SC2; also the complementary sequence from base 1678 to 1695 for both mutants).

RNA (Northern) Blot Analysis. To detect the transcript of the *cotA* gene, RNAs in air-grown cells of *Synechocystis* were extracted by the method of Golden *et al.* (24). Hybridization was carried out with the same probe as used for the plaque hybridization.

Other DNA Methods. Unless otherwise stated, standard techniques were used for DNA manipulation (25). Nucleotides were deleted from the cloned DNA fragment by using a deletion kit (Takara, Tokyo) that contains exonuclease III and mung bean nuclease. Both strands were sequenced with no ambiguities by using a LI-COR DNA analysis system (model 4000).

RESULTS

Growth Responses to CO_2 and Na^+ Concentrations. The SC1 and SC2 mutants grew as fast as the WT in normal BG11 medium with air levels of CO_2 (Fig. 1*A*) or at 3% CO_2 in air (data not shown). These mutants grew much more slowly than the WT when cultures were aerated with 60 ppm CO_2 in CO_2-free air (Fig. 1*B*). It is presumed that under these conditions HCO_3^- transport may not function efficiently and

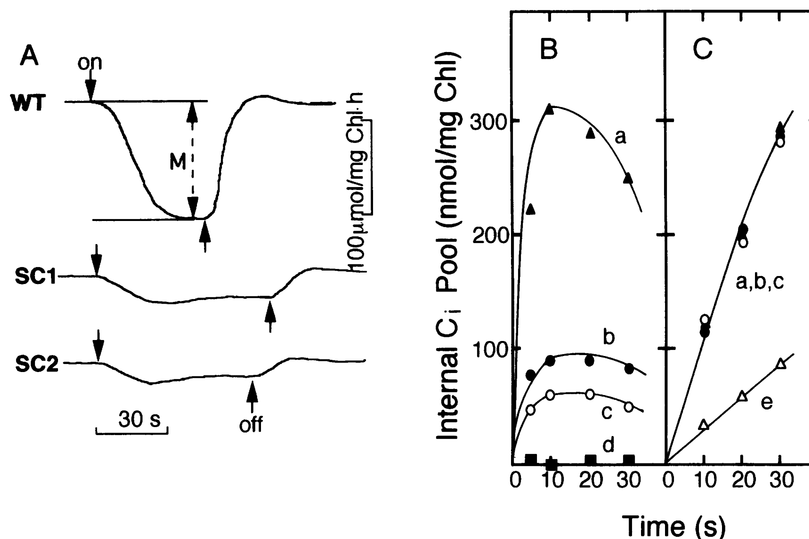

FIG. 2. (*A*) The CO_2 exchange of WT, SC1, and SC2 cells upon switching the light on and off, measured by an open gas analysis system (19). The maximal rate of CO_2 uptake is shown by "M" for WT. (*B* and *C*) Time courses of uptake of $^{14}CO_2$ (*B*) and $H^{14}CO_3^-$ (*C*) into the intracellular C_i pool of WT (curves a and e), SC1 (curves b), and SC2 (curves c and d) cells, measured by the silicone oil-filtering centrifugation method (6). Cells were suspended in 20 mM Hepes-KOH buffer (pH 8.0) containing 15 mM (curves a–d) or 100 μM (curve e) NaCl. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (10 μM) was added to the measurement for curve d. The concentrations of CO_2 and HCO_3^- were 8.8 and 161 μM, respectively.

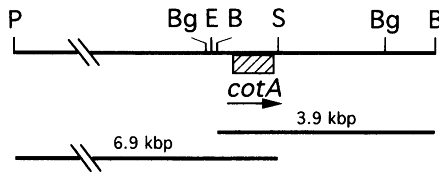

FIG. 3. Restriction map of DNA that complements the mutants. A clone that contains the 3.9-kbp *Bam*HI fragment was isolated by the complementation experiment. The 741-bp nucleotide open reading frame (ORF) (*cotA* gene) is indicated by the hatched bar, with an arrow showing the direction of transcription of the ORF. B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; P, *Pst* I; S, *Spe I.*

that the carbon source is supplied to the cells mainly by CO_2 transport. The slow growth of the mutants was considered to be a result of insufficient supply of carbon because of a defect in the CO_2 transport system. In the low-Na^+ medium, the SC1 mutant grew very slowly, and the SC2 mutant was unable to grow even with air levels of CO_2 (Fig. 1*C*). Since HCO_3^- transport activity is low at 100 μM Na^+ (curve e in Fig. 2*C*), these growth characteristics of the mutants support the view that the mutants are defective in CO_2 transport. The CO_2 transport activity of the SC2 mutant may be too low to support the growth, and contribution of Na^+-independent HCO_3^- transport (26) appears to be negligible in the mutant. At 60 ppm CO_2, the growth of SC2 may be supported by a high-affinity HCO_3^- transport.

Transport of CO_2 and HCO_3^-. The supposition that the mutants have a defect in CO_2 transport was verified by measuring the CO_2 transport activity of the cells. Two methods were applied for this purpose—e.g., an open gas-analysis method (19) and the silicone-oil filtering centrifugation method (6). Fig. 2*A* shows the CO_2 exchange profiles of the cell suspension measured by the gas-analysis system and indicates that the CO_2 uptake activity in SC1 and SC2 was one-third to one-fifth the activity of WT (= M in Fig. 2*A*). The same result was obtained by measuring the uptake of $^{14}CO_2$ into the intracellular C_i pool; the activity of CO_2 uptake was much lower in the mutants than in the WT (curves a–c in Fig. 2*B*). The CO_2 uptake activity of SC2 was lower than that of SC1, suggesting that the mutation in SC1 did not completely abolish the function of the mutated gene. The activity of CO_2 uptake in SC2 was completely abolished by DCMU (curve d in Fig. 2*B*). Thus, the CO_2 uptake in SC2 is not due to diffusion but indicates the presence of CO_2 transport activity in the mutant. The activity was, however, not high enough to support the growth of the mutant (curve c in Fig. 1*C*). There was no difference between the mutants and the WT strains in the activity of $H^{14}CO_3^-$ uptake (curves a–c in Fig. 2*C*). Thus, the CO_2 transport system appears to be specifically impaired in the mutants.

Sequence Analysis of the WT Locus Required for CO_2 Transport. A fraction containing 3- to 4-kbp fragments of the *Bam*HI digest of genomic DNA of WT *Synechocystis* complemented SC1 and SC2 mutants. A clone that complemented the mutants was isolated from a partial genomic library constructed with the above fraction. The clone contained a 3.9-kbp insert DNA (between two *Bam*HI sites in the restriction map shown in Fig. 3). A complementation test with subclones enabled us to map the sites of mutation within a 1.1-kbp nucleotides sequence between the *Bam*HI and *Spe* I sites. Deletion of a 410-bp nucleotide sequence from the *Bam*HI site (to position 1039 in Fig. 4) resulted in a loss of the ability of the clone to complement SC2 mutant but not SC1 mutant. The results indicated that SC1 and SC2 have mutations in different regions. From a phage isolated from a λDASH library that contains DNA of the region contiguous to the above clone, a 6.9-kbp *Pst* I/*Spe* I fragment (left side of the map in Fig. 3) was cloned into pUC18. Sequencing of nucleotides between the *Bam*HI and *Spe* I sites and the upstream region of the *Bam*HI site revealed an ORF consisting of a 1320-bp nucleotide sequence. However, Northern analysis with the 1.1-kbp *Bam*HI/*Spe* I fragment as a probe indicated that the size of the transcript was about 800 bases (Fig. 5*A*). Based on this result, we postulated that ATG at bases 922–924 is the true initiation codon and that the ORF (designated *cotA*) contains 741 base-paired nucleotides and encodes a protein consisting of 247 amino acids. The calculated molecular weight of the protein was 28,547. A Shine–Dalgarno (SD; ref. 27) sequence (GAAG) was found upstream of the postulated initiation codon. A possible promoter sequence can be found upstream of the initiation codon at bases 876–881 (TTTGCC, −35 box) and 897–902 (TACCAT, −10 box). A hairpin structure possibly involved in transcription termination was detected downstream of the *cotA* gene (bases 1679–1708).

Southern hybridization using the *Bam*HI/*Spe* I fragment as a probe showed the presence of a single hybridizing band in each of the *Bam*HI and *Eco*RI digests of the genomic DNA, indicating that the *cotA* gene is present in a single copy (Fig. 5*B*).

Homologous Genes. The amino acid sequence deduced from the nucleotide sequence of the *cotA* gene of *Synechocystis* PCC6803 showed significant similarity to that of the *cemA* gene of pea chloroplasts and its homologues in various plants: liverwort ORF276 (29% identical; refs. 13 and 14), tobacco ORF229 (30%; ref. 15), pea ORF231 (*cemA*, 25%; refs. 12 and 17), and rice ORF230 (24%; ref. 16) (Fig. 6). The *cemA* gene product (CemA) has been immunologically identified in the

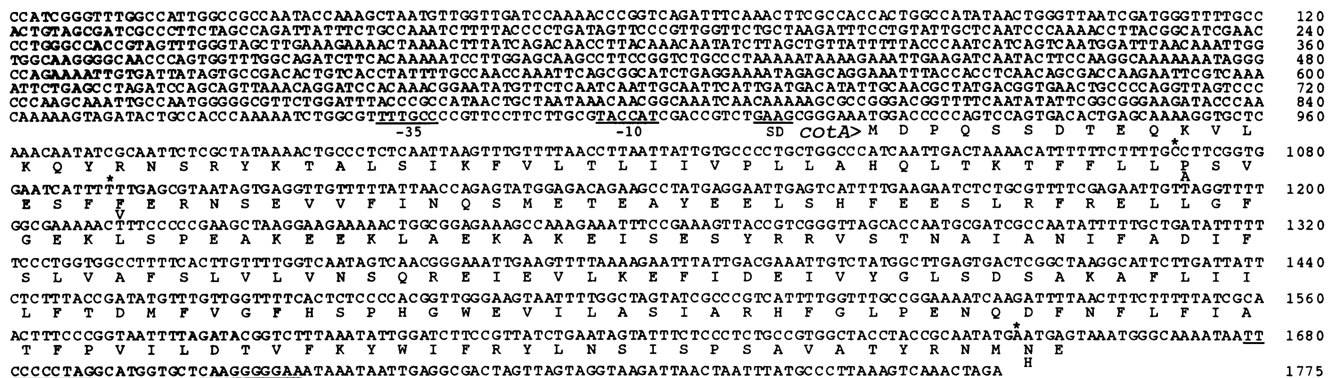

FIG. 4. Nucleotide sequence of DNA in the region of *cotA* and deduced amino acid sequence (single-letter code) of the protein encoded by *cotA*. The asterisk at position 1667 is the base substitution in SC1 (A → C), and those at positions 1072 and 1090 are the C → G and T → G substitutions in SC2. This results in an Asn(N) → His(H) change in SC1 and Pro(P) → Ala(A) and Phe(F) → Val(V) changes in SC2 in the deduced amino acids. The putative promoter sequences (−10 and −35) and the ribosomal binding site (SD) are underlined. An inverted repeat downstream of the *cotA* gene, possibly involved in transcription termination, is also underlined.

FIG. 5. (*A*) Northern blot analysis of total RNA from WT *Synechocystis*. RNA was denatured with formamide, electrophoresed on a 1.2% agarose gel, transferred to nylon membrane, and hybridized with the ^{32}P-labeled 1.1-kbp *Bam*HI/*Spe* I fragment from the *cotA* gene. (*B*) Southern hybridization analysis of genomic DNA from WT *Synechocystis*. DNA (1 μM) digested with *Bam*HI (lane 1) and *Eco*RI (lane 2) was electrophoresed on a 0.8% agarose gel, transferred to nylon membrane (GeneScreen*Plus*, NEN), and then hybridized with the above probe. The sizes of DNA and RNA are shown in kbp.

inner membrane of the chloroplast envelope but not in the thylakoid membrane, which suggested that the *cotA* gene product may be localized in the cytoplasmic membrane.

The function of CemA is not known. Two histidine residues were highly conserved among the plant gene products, but only one of these residues was conserved in *Synechocystis* (asterisk in Fig. 6). The absence of homology between *cotA* and *cemA* products in the region of His-109 in pea (residues in the shadowed box in Fig. 6, which are homologous to the residues containing a putative heme-binding histidine of animal cytochromes and shows the bases in plant genes that encode *b*-type cytochromes; ref. 29) raises a doubt that the *cotA* gene product is a *b*-type cytochrome.

The *cotA* gene product was highly hydrophobic, and the hydropathy index of the protein according to Kyte and Doolittle (30) showed four transmembrane domains; this structure was highly conserved among the products of *cotA*, *cemA*, and the homologous ORFs in chloroplasts of various plants.

Mutations in SC1 and SC2. Sequence analyses of fragments of the respective *cotA* genes of SC1 and SC2 in the region of mutations revealed an A → C change at position 1667 in SC1 and change of two bases (C → G at 1072 and T → G at 1090). The mutations resulted in an Asn-246 → His change in SC1 and Pro-51 → Ala and Phe-57 → Val changes in SC2 in the deduced amino acids (Fig. 4).

DISCUSSION

Mutants SC1 and SC2, which grew very slowly or not at all in low-Na^+ medium, were isolated from *Synechocystis* PCC6803. The growth of these mutants was much slower than that of WT under 60 ppm CO_2 at pH 7.0) (Fig. 1*B*), and complementation tests under these conditions enabled us to clone the gene, designated *cotA*. Evidently, the slow growth of these mutants are the result of the *cotA* mutation. Measurement of CO_2 and HCO_3^- uptake revealed that CO_2 uptake was specifically impaired in the mutants (Fig. 2), indicating that *cotA* encodes a component involved in the CO_2 transport system. The *cotA* mutation in SC1 or SC2, however, did not abolish the CO_2 transport activity completely; about 20–30% of the WT activity remained in the mutants. This suggests the presence of another gene that partially substitutes for the *cotA* function.

Using a mass spectrometric technique, Badger *et al.* have measured both CO_2 and HCO_3^- uptake simultaneously during steady-state photosynthesis (31). They found that some of the characteristics of steady-state C_i transport in cyanobacteria are quite different from those observed where C_i uptake was examined under non-steady-state photosynthesis (8, 31). Yu *et al.* have shown that the affinity of HCO_3^- transport to the substrate in air-grown cells of *Synechococcus* increased when the cells were exposed to 20 ppm of CO_2 and also by changing the external pH from 8.5 to 7.5 (8). These results suggested a possibility that HCO_3^- transport also contributes to the growth of the WT cells at 60 ppm CO_2, pH 7.0. The silicone-oil centrifugation method does not allow us to measure the steady-state activity of HCO_3^- transport and its affinity to the substrate. Therefore, there remains a possibility that the *cotA* mutation not only decreased the CO_2 transport activity but also changed the kinetic parameters and/or Na^+ requirement of the HCO_3^- transport system in the mutants. Detailed study of the steady-state C_i transport will be required to characterize the HCO_3^- transport in the mutants.

Previous studies have shown that C_i transport is driven by NAD(P)H dehydrogenase-dependent photosystem I cyclic electron flow (32, 33). ATP production coupled to the cyclic electron flow is supposed to supply the energy source to drive C_i transport. The *cotA* gene product did not have an ATP binding motif; therefore, it is unlikely that the product is a CO_2 transporter itself energized by ATP. Evidence has accumulated that a vectorial carbonic anhydrase (CA)-like moiety located in the cytoplasmic membrane is involved in the CO_2 transport mechanism. These include the findings that ethoxyzolamide and H_2S, which attack Zn in the reaction center of CA, inhibit CO_2 transport (6, 34, 35). The system would have to depend on metabolic energy supply, since the release of HCO_3^- in the cytoplasm would occur against its chemical potential gradient. The observation that ethoxyzolamide also inhibits HCO_3^- transport suggested that the CA-like moiety is involved in the transport of both C_i species (34). The fact that the *cotA* gene product is homologous to the putative heme-binding proteins suggests that it still has a potential to chelate metal(s), although the heme-binding domain was not conserved in the protein. Thus, the product could be a CA-like protein.

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S6803  MDPQ----------------------------SSDTEQKVLKQYRNSRYKTALSIKFVLTLIIVPLLAHQLTKTFFLLPSVESFFERNSEVVFINQSMETEAYEELSHFEESLRFRELLG
MARPO  :KSSRLTAIAYESIGLVPRSITRTFSRFKAELTNQSSSL:::EF:LAK:QALA:LQYIGC:FFI::GVSFFFQKC::E:WIQNWWNIYQSQI:LTSFQ:EK:LKK:QEI::LFWLDKVMT
TOBAC  :AKKK-----------------------------------------AFTPLFYLASIVFL:WWISFSVNKC-:ESW:TNWWNTGQSEI:L:NIQ:KSLL:KFIEL::L:FLD:MIK
PEA    :AKKK-----------------------------------------AFIPLLCLTSIVFL:WCISFTF:KS-:ESWITHWYNTKESEI:L:TIQ:KSILKKFLE:::LFLLD:M:K
ORIZA  :KKKK-----------------------------------------ALP:FLYLVFIVLL:WGVSFSFNKC-:ELWIKNWWNTRQSQTLLTAIQ:KRVL:RFMEL:DLFILD:MIK

                                                                                            *
       FG-EKLSPEAKEEKLAEKAKEISESYRRVSTNAIANLFADIFSLVAFSLVLVNSQREIEVLKEFIDEIVYGLSDSAKAFLIILFTDMFVGFHSPHGWEVILASIARHFGLPENQDFNFLF
       YSSN:IQLQDLTKEIHQQTI:LVQI:NND:IKIVIHLLT:LIWFITL:CLFILGKERLVI:NSWAQ:LF:S:::TM:::F:L:L::LCI:::::::::IVIS:CLE:::FVH:KHVISC:
       -EYSETHL:EFGIGIHKETIQLIKIQNENRIHT:LHFSTN:IQFIIL:GYSILGNEKLVI:NSWAQ:FL:N:::TV:::S:L:L::LCI:::::::::LMIG::YKD::FVH:DQIISGL
       -EYPETRLQNLRIEIYKETIQLIQTNNQDHIHT:LHFCTN:IQFLIL:VYSIRGNQ:LII:NSWVQ:FL:N:::TI:::S:LFLIEFC::Y::TG:::LMIG:VYKD::FIP:DHIISFL
       -EKPNTHVQNPPIGIRKEIIQLAKIDNEGHLHI:LHFSTN:IQ:AIL:GSFFLGKE:LVI:NSWVQ:FF:N:N::V:::F:L:V::F::::::TR:::LLIRWVYNDL:WVP:ELIFTI:

       IATFPVILDTVFKYWIFRYLNSISPSAVATYRNM--NE-     247
       VS::::::::::::L:::H::R::::I::::HT:--::-     276  (29%)
       VS::::::::I:::::::::::RL:::L:VI:HS:--:D-    229  (30%)
       VSIL:A::::I:::::::::::RV:::L:LI:DSIPFQ:-    231  (25%)
       VCS:::::::CL:F:V:FC::RL:::L:VI:HSIS--:A     230  (24%)
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FIG. 6. Comparison of the deduced amino acid sequences for the products of *cotA* of *Synechocystis*, liverwort(MARPO) ORF276 (13, 14), tobacco(TOBAC) ORF229 (15), pea CemA (12, 17), and rice (Oriza) ORF230 (16). Colons indicate residues of plants identical to corresponding residues in *Synechocystis*. The European Molecular Biology laboratory data base was searched with the computer program developed by Person and Lipman (28). The shadowed box indicates residues surrounding the putative heme-binding histidine. The asterisk indicates the histidine residue conserved among the products of the *Synechocystis* and plant genes.

It is also possible that the *cotA* gene product has a role in a number of physiological reactions including CO_2 transport, and the growth characteristics of the mutants reflect all of these reactions. If this is the case, the *cotA* gene product will have a function not specialized to CO_2 transport. Then, the product could play a role in proton pumping (e.g., extruding proton produced as a result of CO_2-to-HCO_3^- conversion), or it could be a component required to energize systems such as the CO_2-to-HCO_3^- converting system or the HCO_3^--to-CO_2 converting system in the front-end model (34). Further study is necessary to elucidate the exact function of the gene product in CO_2 transport.

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