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Biosynthesis of a 42-kD Polypeptide in the Cytoplasmic Membrane of the Cyanobacterium *Anacystis nidulans* Strain R2 during Adaptation to Low CO_2 Concentration[1]

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ABSTRACT

When cells of *Anacystis nidulans* strain R2 grown under high CO_2 conditions (3%) were transferred to low CO_2 conditions (0.05%), their ability to accumulate inorganic carbon (C_i) increased up to 8 times. Cytoplasmic membranes (plasmalemma) isolated at various stages of low CO_2 adaptation were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. There was a marked increase of a 42-kilodalton polypeptide in the cytoplasmic membrane during adaptation; a linear relationship existed between the amount of this polypeptide and the C_i-accumulating capability of the cells. No significant changes were observed during this process in the amount of other polypeptides in the cytoplasmic membranes or in the polypeptide profiles of the thylakoid membranes, cell walls, and soluble fractions. Spectinomycin, an inhibitor of protein biosynthesis, inhibited both the increase of the 42-kilodalton polypeptide and the induction of high C_i-accumulating capability. The incorporation of [^{35}S]sulfate into membrane proteins was greatly reduced during low CO_2 adaptation. Radioautograms of the ^{35}S-labeled membrane proteins revealed that synthesis of the 42-kilodalton polypeptide in the cytoplasmic membrane was specifically activated during the adaptation, while that of most other proteins was greatly suppressed. These results suggested that the 42-kilodalton polypeptide in the cytoplasmic membrane is involved in the active C_i transport by *A. nidulans* strain R2 and its synthesis under low CO_2 conditions leads to high C_i-transporting activity.

Cyanobacteria have a mechanism to transport exogenous C_i[2] and concentrate it internally (1, 7, 13, 16, 20, 27). The CO_2-concentrating mechanism involves an active C_i transport, which is driven by PSI reaction of photosynthesis (20–22). While the presently available evidence does not allow one to explain the transport with a specific mechanism, three models are most commonly discussed: (a) a primary electrogenic HCO_3^- pump (9), (b) H^+/HCO_3^- symport or OH^-/HCO_3^- antiport secondary to an H^+ extrusion pump (4, 16), and (c) Na^+/HCO_3^- symport secondary to a Na^+/H^+ exchange (8, 17). Any of these mechanisms of C_i transport would require the participation of special protein(s) in the cytoplasmic membrane. Investigations of such protein(s) are an important first step in the elucidation of the mechanism of C_i transport.

Exposure of high CO_2-grown cells of cyanobacteria to low CO_2 conditions increases their C_i-accumulating capability and their photosynthetic affinity to C_i (15, 25, 28). Since it is already known that protein synthesis is required for induction of this C_i-accumulating capability (15), a protein(s) involved in the C_i-transporting mechanism appears to be synthesized during low CO_2 adaptation. Two kinds of proteins have been reported to increase during this process. One is CA found in *Coccochloris peniocystis* (6) and three strains of *Anabaena variabilis* (28, 30). The other is a 42-kD polypeptide in the cytoplasmic membrane of *Anacystis nidulans* (Richt.) Drouet and Daily (originally derived from Tx20 of J. Myers and hereafter referred to str. Tx20) (25). The content of the latter protein was much higher in low CO_2-adapted cells than in high CO_2-grown cells.

In this study a 42-kD polypeptide was also found in the cytoplasmic membrane of *A. nidulans* str. R2. We describe the time course for the increase of the 42-kD polypeptide during exposure of high CO_2-grown cells to low CO_2 conditions and compare it with the time course for induction of their C_i-accumulating capability. We further investigated the incorporation of [^{35}S]sulfate into this polypeptide during low CO_2 adaptation. Our results demonstrated a close correlation between the amount of the 42-kD polypeptide and C_i-accumulating capability of the cells, and a specific activation of biosynthesis of this polypeptide during the low CO_2 adaptation.

MATERIALS AND METHODS

Growth of *Anacystis* and Exposure to Low CO_2 Conditions. *Anacystis nidulans* str. R2 was kindly provided by Dr. L. A. Sherman (Division of Biological Sciences, University of Missouri, Columbia, MO) and grown at 30°C in BG-11 medium (29) under aeration with 3% CO_2 in air. Continuous illumination was provided by fluorescent lamps at 120 μmol PAR/m^2·s. For low CO_2 adaptation experiments, cells were harvested by centrifugation at 2200*g* for 10 min, resuspended in fresh BG-11 growth medium supplemented with 20 mM Hepes-NaOH buffer (pH 7.0), and aerated with air containing about 0.05% CO_2 under the same light conditions.

Gas Exchange Measurements. Cells were harvested by centrifugation at 3000*g* for 5 min, resuspended in 30 ml of 40 mM Hepes-NaOH buffer (pH 7.0) at a Chl concentration of 4 to 6 μg/ml and then placed in a reaction vessel (23). The gas exchange of the cells in the reaction vessel was measured using an open gas analysis system under N_2 containing 200 μl CO_2/L (22, 23). The amount of C_i accumulated within the cells was calculated from the total amount of CO_2 evolved as the burst in the dark after 5 min of illumination with orange light (≥560 nm, 1.3 mE/

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[2] Abbreviations: C_i, inorganic carbon; CA, carbonic anhydrase; C_0, intracellular concentration of inorganic carbon; SIS, sorbitol impermeable space; CBB, Coomassie Brilliant Blue R-250.

$m^2 \cdot s$); the postillumination CO_2 burst was measured in the presence of 3 mM iodoacetamide which inhibits photosynthetic CO_2 fixation completely (3, 21). It has been shown that the amount of intracellular C_i calculated by the CO_2 burst method agrees with that measured using the silicone oil-centrifugation method (23). The intracellular C_i concentration (C_0) was calculated using the SIS value (5) as the intracellular water space (7, 16, 27).

Preparation of Cytoplasmic Membranes, Thylakoid Membranes, Cell Walls, and Soluble Fractions. Cytoplasmic and thylakoid membranes were prepared by a modification of the method described by Omata and Murata (24). The cells amounting to about 0.5 ml packed cell volume were suspended in 20 ml of 5 mM Tes-NaOH buffer (pH 7.0) containing 0.6 M sucrose and 2 mM EDTA and incubated with 6 mg lysozyme at 30°C for 2 h. The lysozyme-treated cells were collected by centrifugation at 8000*g* for 5 min, washed with 20 mM Tes-NaOH buffer (pH 7.0) containing 0.6 M sucrose, suspended in 10 ml of the same buffer, and then disrupted by passing through a French pressure cell at 80 MPa. After addition of 0.0001% DNase I (Sigma, DN-EP) and 1 mM each of phenylmethylsulfonyl fluoride, ϵ-aminocaproic acid, and benzamidine, the homogenate was centrifuged at 8,000*g* for 10 min to remove unbroken cells. The supernatant was made up to a sucrose concentration of 50% (w/v) by adding 0.74 volume of 90% sucrose solution. A 17-ml aliquot was placed at the bottom of a 35-ml centrifuge tube, overlaid with 8 ml of 39%, 3 ml of 30%, and 7 ml of 10% sucrose solutions (w/v), and centrifuged at 130,000*g* for 16 h at 4°C in a swinging bucket rotor (Hitachi RPS 27-2). All the sucrose solutions contained 10 mM Tes-NaOH buffer (pH 7.0), 10 mM NaCl, and 5 mM EDTA. Cytoplasmic membranes formed a band in the 30% sucrose layer and thylakoid membranes at the interface between the 39 and 50% sucrose layers. Cell walls were pelleted at the bottom. The cytoplasmic membranes and thylakoid membranes were withdrawn from the gradient and collected by centrifugation at 300,000*g* for 1 h after 3-fold dilution with 10 mM Tes-NaOH buffer (pH 7.0) containing 10 mM NaCl. Cell walls were purified from the sediment; the sediment was resuspended in 5 mM Tes-NaOH buffer (pH 7.0), and centrifuged on a continuous sucrose density gradient according to Murata *et al.* (19). Soluble fractions of ^{35}S-labeled cells were prepared from the 50% sucrose layer. The layer was withdrawn from the gradient, diluted 3-fold with the Tes-NaOH buffer containing NaCl, and centrifuged at 300,000*g* for 1 h to remove residual thylakoid membranes. The supernatant from this centrifugation was used as soluble fraction. For the preparation of soluble fractions from unlabeled cells, intact cells (0.5 ml packed volume) were suspended in 10 ml of 10 mM Tes-NaOH buffer (pH 7.0) containing 1 mM each of the protease inhibitors and 0.0001% DNase I and disrupted by passing through a French pressure cell at 150 MPa. The supernatant obtained after centrifugation at 300,000*g* for 2 h of the homogenate was used as soluble fraction.

The Chl contents of the cytoplasmic membrane preparations ranged from 10 to 17 μg/mg protein, while those of the thylakoid membrane preparations were about 130 μg/mg protein. Assuming that Chl is exclusively localized in the thylakoid membranes, we estimated the level of contamination of the cytoplasmic membrane preparations by the thylakoid membranes to be less than 13% on protein basis.

SDS-PAGE. This was performed in the buffer system of Laemmli (11). The samples were solubilized in 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 10% glycerol, 10% mercaptoethanol, and 0.001% bromophenol blue. Cell walls were solubilized at 100°C for 5 min, whereas the other samples were solubilized at room temperature for 30 min. The stacking gel contained 5% polyacrylamide, and the separating gel contained a linear gradient of polyacrylamide concentration from 8 to 15%. Each sample was applied to a well of 0.7 cm width on a gel of 0.1 cm thickness. Electrophoresis was carried out at room temperature. After the electrophoresis, gels were stained with CBB and densitometry was carried out by measuring *A* at 560 nm with 750 nm as a reference beam using a dual-wavelength densitometer (Shimadzu, CS-900) with a chromatographic data processing computer (Shimadzu, C-R3A). The content of 42-kD polypeptide in cytoplasmic membranes was shown by the area under the 42-kD polypeptide peak relative to the total area under all the peaks.

Labeling with ^{35}S. Protein biosynthesis by *A. nidulans* str. R2 was investigated by *in vivo* labeling of the cells with ^{35}S. For this experiment, high CO_2-grown cells were harvested by centrifugation at 2,200*g* for 10 min and resuspended in fresh growth medium containing one-tenth the normal concentration of sulfate (0.03 mM) and 20 mM Hepes-NaOH buffer (pH 7.0) at a Chl concentration of about 5 μg/ml. The cell suspension was divided into two portions, one of which was aerated with air (0.05% CO_2) and the other with 3% CO_2 in air. After 3.5 h of incubation in the light, $Na_2{}^{35}SO_4$ (Amersham) was added to these cultures to a concentration of 5 μCi/ml (final sulfate concentration = 0.1 mM). The cells were harvested after 2.5 h of labeling in the light and subjected for analysis.

Radioautograms of ^{35}S-Labeled Polypeptides. After SDS-PAGE of ^{35}S-labeled samples, gels were stained, photographed using Polaroid 665 film, incubated in EN^{3}HANCE (New England Nuclear) and then dried on a filter paper. Radioautograms were obtained by exposing the dried gels to Kodak X-Omat AR-5 film at - 70°C for 3 to 7 d.

Protein and Chl Analyses. Amount of protein was determined according to Lowry *et al.* (12), and that of Chl according to Mackinney (14).

RESULTS

Accumulation of C_i following Exposure of High CO_2-Grown Cells to Low CO_2 Conditions. The C_i-accumulating capability of high CO_2-grown cells of *A. nidulans* str. R2 is affected by the length of exposure to low CO_2 conditions. The intracellular C_i concentration at steady state in the light (C_0) of high CO_2-grown cells was 10 mM and was increased to 83 mM after 8 h of low CO_2 incubation (Fig. 1B). The Chl content of the culture remained almost constant during this period, whereas SIS of the cells increased by 60% from 80 μl/mg Chl to 139 μl/mg Chl (Fig. 1A). The C_0 value of high CO_2-grown cells and the maximal C_0 value after low CO_2 adaptation were almost constant in induction experiments with different cultures, but the time courses of adaptation were variable. The time required for maximal adaptation varied between 6 and 10 h. Time courses for induction of C_i-transporting activity have been reported on *Anabaena variabilis*, strains M3 (15) and ATCC 29413 (28) and *A. nidulans* str. Tx20 (25). In the case of the two strains of *A. variabilis*, the adaptation observed as increases in apparent photosynthetic affinity of the cells to C_i was almost complete after 4 h of exposure to low CO_2 conditions.

Changes in Polypeptide Compositions during Low CO_2 Adaptation. Cytoplasmic membranes, thylakoid membranes, cell walls and soluble fractions were prepared from *Anacystis* cells at various stages of low CO_2 adaptation and their polypeptide compositions were analyzed by SDS-PAGE. The cells used for these preparations were obtained from the same culture used in the measurements for Figure 1, A and B. The polypeptide compositions of the cytoplasmic (Fig. 2, lane a) and thylakoid (Fig. 3, lane a) membranes of high CO_2-grown cells were essentially the same as those, respectively, reported previously for *A. nidulans* str. Tx20 (25). The polypeptide profiles of cytoplasmic membranes obtained at various stages of low CO_2 adaptation indicate that only a 42-kD polypeptide increased during this

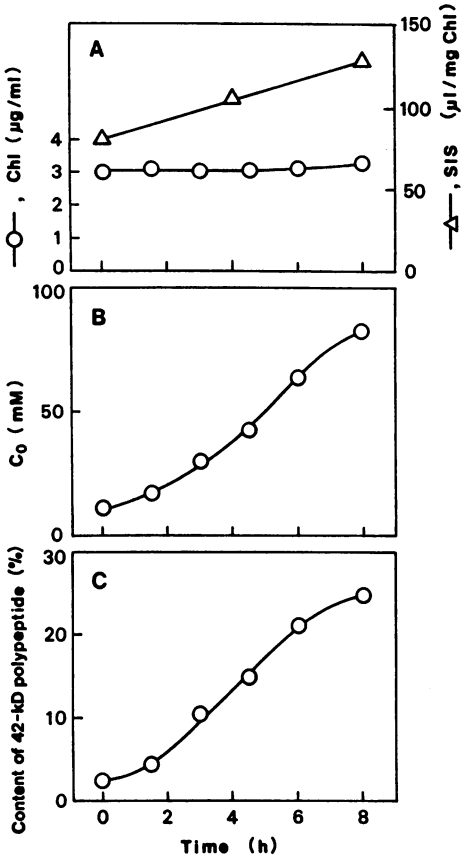

FIG. 1. Time courses of changes of Chl content (A), SIS (A), C_0 (B) and the content of the 42-kD polypeptide in the cytoplasmic membrane (C) after high CO_2-grown cells were transferred to low CO_2 conditions at time zero.

process (Fig. 2, lanes a–f). Calculation on the densitometric traces of the stained gel showed that the content of this polypeptide was 3% of the total proteins in the cytoplasmic membrane from high CO_2-grown cells, and increased progressively during the adaptation to reach 25% after 8 h (Fig. 1C). There existed a linear relationship between the content of the 42-kD polypeptide and the C_i-accumulating capability of the cells (Fig. 4).

In contrast to the drastic changes occurring in the cytoplasmic membranes, only small changes were observed in polypeptide compositions of the thylakoid membranes during low CO_2 adaptation (Fig. 3, lanes a and b). These include an increase of 27-, 42-, and 72-kD polypeptides. Since the increase of the 27- and 72-kD polypeptides was almost negligible in the thylakoid membrane from *A. nidulans* str. Tx20 during low CO_2 adaptation (25), these polypeptides do not seem to be essential in low CO_2 adaptation. The polypeptide profiles of the thylakoid membranes from high CO_2-grown (Fig. 3, lane a) and low CO_2-adapted (lane b) cells showed a band at 37-kD, the molecular mass identical with that of the major polypeptide in the cytoplasmic membranes. The ratios in content of the 42-kD polypeptide to that of the 37-kD polypeptide in the thylakoid membranes from low CO_2-adapted cells was approximately the same as that in the cytoplasmic membrane. Thus, the 42-kD and 37-kD polypeptides observed in the thylakoid membranes seem to be due to contamination by cytoplasmic membranes. Assuming that the 37-kD polypeptide is solely associated with the cytoplasmic membrane, we estimated the level of contamination of the thylakoid membrane preparation by the cytoplasmic membranes to be less than 10% on protein basis.

The cell walls contained major polypeptides with apparent

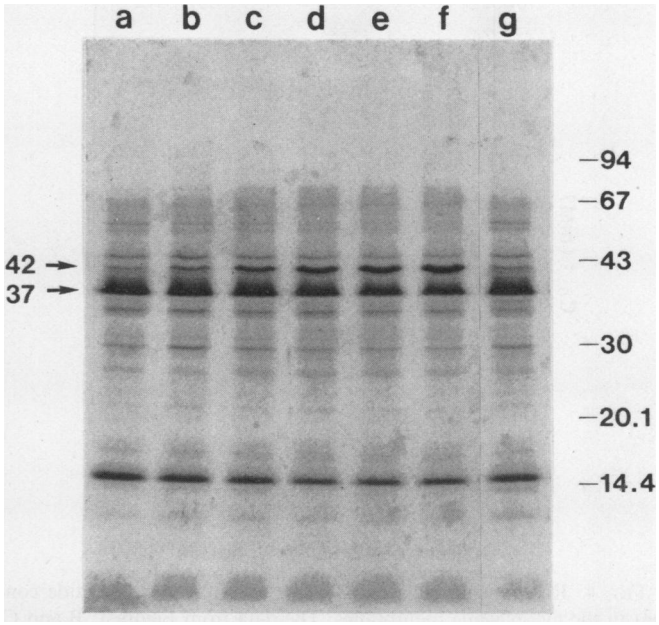

FIG. 2. Electrophoretic profiles showing CBB-staining patterns of polypeptides in the cytoplasmic membranes from high CO_2-grown cells before (a) and after 1.5 h (b), 3 h (c), 4.5 h (d), 6 h (e), and 8 h (f) of exposure to low CO_2 conditions. Lane g shows a polypeptide pattern of the cytoplasmic membrane from the cells exposed to low CO_2 conditions for 6 h in the presence of spectinomycin (10 μg/ml). The photograph of a dried gel is shown. Each sample applied to the gel contained 10 μg protein. The numbers to the right represent the position of marker proteins, *i.e.* α-lactalbumin (14.4 kD), a trypsin inhibitor (20.1 kD), carbonic anhydrase (30 kD), ovalbumin (43 kD), albumin (67 kD), and phosphorylase b (94 kD).

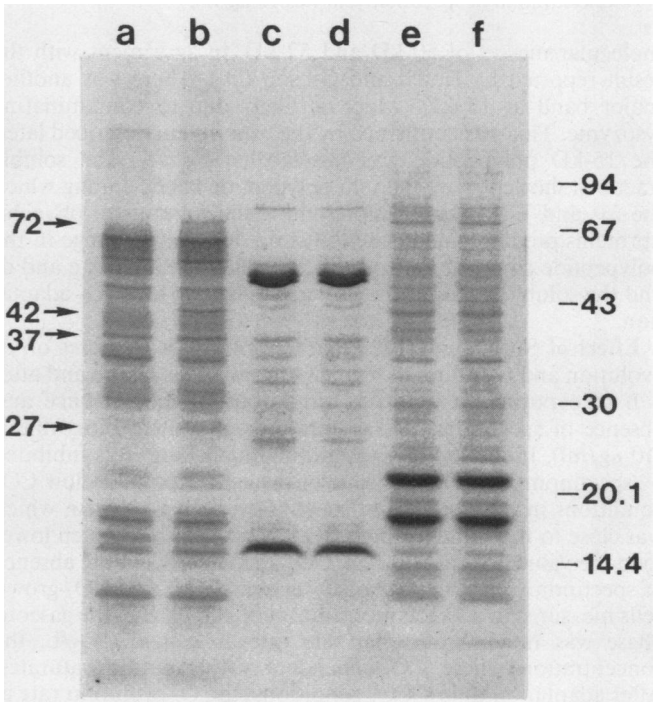

FIG. 3. Electrophoretic profiles showing CBB-staining pattern of polypeptides in the thylakoid membranes (a and b), cell walls (c and d), and soluble fractions (e and f) from high CO_2-grown cells before (a, c and e) and after 8 h (b, d and f) of adaptation to low CO_2 conditions. Other conditions as in Figure 2.

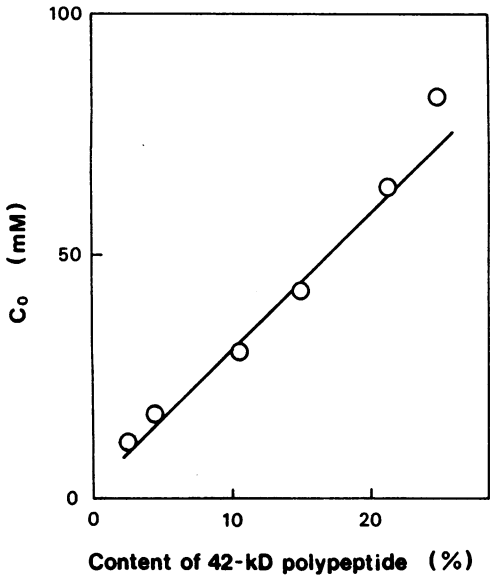

FIG. 4. Relationship between C_0 value and 42-kD polypeptide content in the cytoplasmic membranes. The data from Figure 1, B and C, were replotted.

Table I. *Effect of Spectinomycin on Induction of C_i-Accumulating Capability of Anacystis Cells by Low CO_2 Concentration*

Incubation Conditions	O_2 Evolution		C_0
	800 μl CO_2/L	200 μl CO_2/L	
	μmol/mg Chl·h		*mM*
High CO_2	153	89	9
Low CO_2, 6 h	158	158	51
Low CO_2 + spectinomycin[a], 6 h	140	104	11

[a] Concentration of spectinomycin was 10 μg/ml.

molecular masses of 50 kD and 52 kD, in agreement with the result reported by Resch and Gibson (26). There was another major band at 15 kD, which is likely due to contaminating lysozyme. This was confirmed by the experiment described later; the 15-kD polypeptide was not labeled by ^{35}S. The soluble fractions showed more than 40 polypeptide bands among which the 21- and 17-kD bands representing the subunits of phycobiliproteins predominated. There was no detectable change in the polypeptide composition in the cell walls (Fig. 3, lanes c and d) and the soluble fractions (lanes e and f) during low CO_2 adaptation.

Effect of Spectinomycin. Table I summarizes the rates of O_2 evolution and C_0 values of high CO_2-grown cells before and after 6 h of exposure to low CO_2 conditions in the presence and absence of spectinomycin, an inhibitor of protein biosynthesis (10 μg/ml). Induction of C_i-accumulating activity was inhibited by spectinomycin. The C_0 value of the cells exposed to low CO_2 conditions in the presence of spectinomycin was 11 mM, which was close to the value for high CO_2-grown cells and much lower than the value obtained for low CO_2-adapted cells in the absence of spectinomycin. The rate of O_2 evolution of high CO_2-grown cells measured at a CO_2 concentration of 200 μl/L in the gaseous phase was much lower than the rate at 800 μl CO_2/L, the concentration where CO_2-dependent O_2 evolution saturates. After adaptation to low CO_2 conditions, the O_2 evolution rate at 200 μl CO_2/L had risen to a value equal to that at 800 μl CO_2/L, indicating that their efficiency of C_i assimilation was increased. This can be attributed to the higher C_i-accumulating capability. Inhibition of induction of C_i-accumulating capability by spectinomycin was reflected by a lower rate of O_2 evolution at 200 μl CO_2/L as compared with that at 800 μl CO_2/L even after exposure of the cells to low CO_2 conditions. Since spectinomycin inhibited the O_2-evolution activity at 800 μl CO_2/L only by 10%, the lower C_0 value of the spectinomycin-treated cells as compared with that of the untreated cells was not due to inhibition of photosynthesis but due to inhibition of protein synthesis. Hence, our results indicated that *de novo* protein synthesis is necessary for adaptation of high CO_2-grown cells to low CO_2 conditions, being consistent with the results reported with *A. variabilis* M3 (15).

As expected, the polypeptide profile of the cytoplasmic membrane prepared from spectinomycin-treated cells (Fig. 2, lane g) showed no increase of the 42-kD polypeptide even after 6 h of exposure to low CO_2 conditions.

Biosynthetic Incorporation of ^{35}S into Polypeptides. To directly monitor the *de novo* synthesis of proteins, high CO_2-grown cells were exposed to high and low CO_2 conditions, respectively, for 3.5 h in the light and then incubated with $Na_2{}^{35}SO_4$ for 2.5 h under the same conditions. The C_0 value of the cells exposed to low CO_2 conditions for 3.5 h was 19 mM and was increased to 36 mM after 2.5 h of labeling period. Table II summarizes the total amount of ^{35}S incorporated into the membranes, cell walls, and soluble fractions during the labeling period under high and low CO_2 conditions, or shows that the incorporation of ^{35}S into these preparations is much less under low CO_2 conditions.

The electrophoretic profile of the ^{35}S-labeling pattern of polypeptides in the cytoplasmic membrane from the cells labeled under high CO_2 conditions (Fig. 5, lane Ac) was very similar to the corresponding CBB-staining pattern (Aa). Both patterns showed a major band at 37 kD. In contrast, the cytoplasmic membrane from the cells labeled under low CO_2 conditions incorporated most of the label into the 42-kD polypeptide (Ad). This polypeptide became much more strongly labeled than in the cells growing under high CO_2 conditions (Ac), although the total amount of ^{35}S incorporated into cytoplasmic membrane was about 12-fold smaller under the low CO_2 conditions (Table II). Apparently, the biosynthesis of the 42-kD polypeptide was specifically activated during low CO_2 adaptation, while that of all other polypeptides in the cytoplasmic membrane was suppressed. Our data revealed that the increase of the 42-kD polypeptide during low CO_2 adaptation was not due to degradation or conversion of other proteins but due to *de novo* synthesis.

The ^{35}S-labeling pattern of polypeptides in the thylakoid membrane from the cells labeled under high CO_2 conditions (Fig. 5, lane Bc) was similar to the corresponding staining pattern (Ba) except that a polypeptide band with apparent molecular mass of 32 kD was particularly strongly labeled. This band may represent the herbicide binding protein of PSII which is rapidly turned over (10). The 32-kD band was the most prominent one in the ^{35}S-labeling pattern of polypeptides in the thylakoid membranes from the cells labeled under low CO_2 conditions (Bd). The incorporation of ^{35}S into this band, however, was much less under low CO_2 conditions than that under high CO_2 conditions (Bc and Bd). The ^{35}S-labeling pattern of polypeptides in the cell walls and soluble fractions from the cells labeled under high CO_2 conditions (Cc and Dc) were similar to the corresponding staining

Table II. *Incorporation of [^{35}S]Sulfate into Cytoplasmic and Thylakoid Membranes, Cell Walls, and Soluble Fractions of Anacystis Cells under High and Low CO_2 Conditions*

Labeling Conditions	Incorporation of ^{35}S			
	Cytoplasmic membrane	Thylakoid membrane	Cell wall	Soluble fraction
	cpm/μg protein			
High CO_2, 2.5 h	3400	1200	290	540
Low CO_2, 2.5 h	270	150	70	100

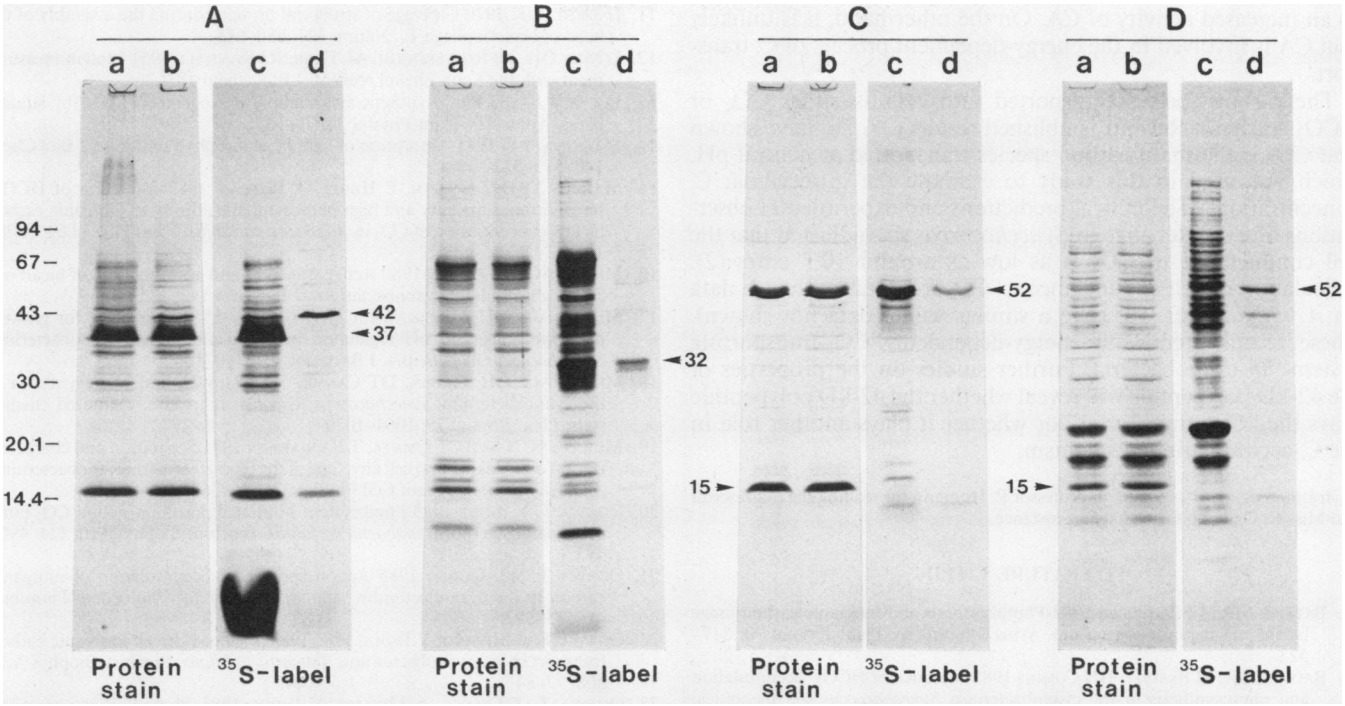

FIG. 5. Electrophoretic profiles showing ^{35}S-labeling pattern (c and d) and CBB-staining pattern (a and b) of polypeptides in the cytoplasmic membranes (A), thylakoid membranes (B), cell walls (C), and soluble fractions (D) from *Anacystis* cells labeled under high (a and c) and low CO_2 conditions (b and d). Each sample applied to the gel contained 25 μg protein for A, B, and D, and 15 μg protein for C. The radioautograms were obtained by exposing the dried gel to x-ray film for 3 d (A and B) or 7 d (C and D). The numbers to the left of lane Aa represent the position of the marker proteins.

patterns (Ca and Da, respectively), except that no label was found in the band at 15 kD which is one of the major bands in the staining patterns. As suggested already above, this 15-kD polypeptide probably represents the contamination by lysozyme used for digestion of peptidoglycan. There was no polypeptide actively synthesized in cell walls during low CO_2 adaptation (Cd). In low CO_2-adapting cells, synthesis of most of the polypeptides in the soluble fractions was suppressed, whereas synthesis of the 52-kD polypeptide was sustained (Dd).

The electrophoretic analysis of the ^{35}S-labeled membranes revealed that a large amount of label was incorporated into lipid fraction of the cytoplasmic membrane from the cells labeled under high CO_2 conditions (Fig. 5, lane Ac). This probably represents the incorporation of ^{35}S into sulfoquinovosyl diacylglycerol, one of the major lipid classes in cyanobacteria. Incorporation of ^{35}S into lipid was strongly suppressed during low CO_2 adaptation (Ad). Thylakoid membranes incorporated only a small amount of ^{35}S into lipid even under the high CO_2 conditions (Bc). The content of sulfoquinovosyl diacylglycerol in the cytoplasmic and thylakoid membranes from high CO_2-grown cells of *A. nidulans* str. Tx20 was 100 μg/mg protein and 30 μg/mg protein, respectively (24). The differences in the content of this lipid may explain the higher incorporation of label into lipid fraction of the cytoplasmic membrane. There is a possibility, however, that the biosynthetic activity of sulfoquinovosyl diacylglycerol is much higher in the cytoplasmic membrane.

DISCUSSION

Exposure of high CO_2-grown cells of cyanobacteria to low CO_2 conditions increases their C_i-transporting activity and apparent photosynthetic affinity to C_i (7, 15, 25, 28). Being sensitive to spectinomycin, the adaptation process appears to involve protein synthesis (Table I, also see ref. 15). A previous study with *A. nidulans* str. Tx20 has shown that the cytoplasmic membrane from low CO_2-adapted cells contains a much higher amount of a 42-kD polypeptide than that from high CO_2-grown cells (25). Using *A. nidulans* str. R2, the present study clearly demonstrated that it is specifically the biosynthesis of the 42-kD polypeptide in the cytoplasmic membrane which is stimulated during low CO_2 adaptation (Fig. 5, lanes Ac and Ad). Accordingly, the inhibitory effect of spectinomycin on the adaptation process could be traced directly to a prevention of the synthesis of this one polypeptide. Our results strongly suggest, therefore, that the 42-kD polypeptide in the cytoplasmic membranes is involved in the C_i-transporting mechanism and that the stimulation of its synthesis under low CO_2 conditions induces high C_i-accumulating capability of the cells.

Investigations on another cyanobacterium, *A. variabilis*, have identified CA as a protein that appears to be synthesized during adaptation to low CO_2 conditions (28, 30). Activity of this enzyme could be demonstrated only after disruption of the cells, indicating that it is not present in the cell wall or in the cytoplasmic membrane. Similarly, no CA activity was found with intact cells of *A. nidulans* str. Tx20 (18). Assuming the same situation to prevail in our strain of *A. nidulans*, the 42-kD polypeptide cannot be CA. There was, however, a slight increase of 27- and 72-kD polypeptides in the thylakoid membranes during low CO_2-adaptation (Fig. 3, lanes a and b). One of these polypeptides possibly could be CA.

In *A. variabilis* ATCC 29413, the CA activity increased during low CO_2 adaptation in parallel with a decrease of the K_m ($NaHCO_3$) of photosynthetic O_2 evolution of the cells (28). A mass spectrometric measurement of $C^{16}O_2$ evolved in the light from low CO_2-grown cells of *Synechococcus* sp. in the presence of $NaHC^{18}O_3$ in the external medium indicated that CA functioned in supplying CO_2 from HCO_3^- within the cells (2). Consequently, one could at least partly ascribe the decrease of K_m ($NaHCO_3$) value of photosynthetic O_2 evolution during low CO_2 adaptation

to an increased activity of CA. On the other hand, it is unlikely that CA is involved in the energy-dependent process of C_i transport.

The carbon species transported into cells is either CO_2 or HCO_3^- or both. Recently published results (27, 28) have shown that CO_2 is the main carbon species transported at neutral pH, which was used in this study to estimate the intracellular C_i concentrations. Theoretical predictions and experimental observations of CO_2 exchange in *Synechococcus* sp. indicated that the cell conductance to CO_2 is as low as around 10^{-5} cm/s (2). Calculation of cell conductance to CO_2 from gas-exchange data on *A. nidulans* str. R2 gave a similar value (data not shown). These results predict an energy-dependent "CO_2-transporting system" in cyanobacteria. Further studies on the properties of the 42-kD polypeptide will reveal whether the 42-kD polypeptide plays the "CO_2 transporter" or whether it plays another role in the C_i-accumulating mechanism.

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