Plant Physiol. (1987) 84, 633–639
0032-0889/87/84/0633/07/$01.00/0

Isolation and Characterization of a Carotenoid-Associated Thylakoid Protein from the Cyanobacterium *Anacystis nidulans* R2[1]

Received for publication January 30, 1987 and in revised form March 19, 1987

KAZUMORI MASAMOTO[2], HAROLD C. RIETHMAN, AND LOUIS A. SHERMAN*
Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

ABSTRACT

A carotenoid-associated membrane protein was isolated from *Anacystis nidulans* R2 thylakoids. Sodium pyrophosphate and sodium bromide washed thylakoids were solubilized with the nonionic detergents dodecyl-β-D-maltoside and octyl-β-D-glucopyranoside, and these detergent extracts were fractionated on a sucrose density gradient. A yellow fraction from the sucrose gradient was further purified by anion-exchange and organomercuric-affinity column chromatography to yield a fraction virtually free of chlorophyll and highly enriched in both carotenoids and a 42 kilodalton polypeptide. Evidence presented in this paper suggests that the carotenoid-containing 42 kilodalton protein is thylakoid associated rather than cytoplasmic membrane associated. The purified 42 kilodalton polypeptide was used to raise polyclonal antibodies in rabbits. Immunochemical detection of the 42 kilodalton polypeptide on Western blots demonstrated an increased accumulation of this polypeptide in cells grown under high-light conditions relative to cells grown under low light.

Cyanobacteria have three distinct membrane systems; outer and inner (cytoplasmic) membranes comprise the cell envelopes, as in other gram-negative bacteria (8), whereas the internal thylakoid membrane system contains the components responsible for an oxygen-evolving electron transfer system unique among prokaryotes (10). Using improved isolation techniques, envelope (outer and cytoplasmic) membranes have been separated from thylakoids in both *Anacystis nidulans* (11, 22, 24, 27) and *Synechocystis* PCC6714 (10, 11, 17, 18), and have been shown to contain carotenoids but little or no Chl. The major carotenoids of the isolated outer and cytoplasmic membranes were xanthophylls, whereas the major thylakoid-associated carotenoid was β-carotene (11, 17, 18, 22, 24, 25, 27).

Analysis of *A. nidulans* membrane components involved in photosynthesis has yielded important information regarding integral membrane proteins that are stably liganded to Chl (3, 26). These Chl-binding proteins include the PSI Chl-protein and oligomers CP I, CP II, CP III, and CP IV (3), and the PSII-associated Chl-proteins CP VI-2 (spinach chloroplast CP 47 analog), CP VI-3 (spinach chloroplast CP 43 analog) and CP VI-4 (a Chl-protein synthesized in response to iron stress). An additional PSII-associated Chl-protein, CP VI-1, contains both the 71 kD phycobilisome 'anchor' polypeptide and somewhat variable amounts of an unidentified 42 kD polypeptide (26; HC Riethman, LA Sherman, unpublished data). The presence of carotenoids in the photosynthetic membranes of *A. nidulans* is likely to be physiologically important both for the protection of these Chl-proteins from photooxidative damage and for the harvesting of light energy in spectral regions where Chl *a* and phycobilisomes do not absorb strongly.

Although carotenoids have been found as ligands on many isolated Chl-binding proteins (32), there have been few reports of carotenoids specifically associated with isolated proteins in the absence of Chl. Holt and Krogmann (14) reported on a carotenoid-binding protein isolated from aqueous extracts of several cyanobacteria, whereas Bullerjahn and Sherman (6) have isolated an integral membrane, carotenoid-associated protein from cytoplasmic membranes of *Synechocystis* PCC 6714. The carotenoid-associated protein from *Synechocystis* PCC 6714 was shown to have an immunologically related analog in *A. nidulans* R2 (5, 6). In this study we describe the isolation and characterization of a second carotenoid-associated protein from *A. nidulans* R2, one that is immunochemically distinct from that described previously (5, 6). This protein appears to be embedded in the thylakoid membrane, and accumulates specifically in cells grown under high light conditions.

MATERIALS AND METHODS

Cell Growth. *Anacystis nidulans* R2 was grown in BG-11 medium with constant illumination (0.5 mW/cm^2) and aeration at 25°C in 15 L carboys as described previously (12). Growth under high white light and low white light were at intensities of 1.9 mW/cm^2 and 0.02 mW/cm^2, respectively. For preparation of thylakoids from iron-deficient cells, cells were grown in low-iron medium as described earlier (13, 29).

Isolation of Thylakoid Membranes and the Carotenoid-Associated Membrane Protein Fraction. Thirty L of *A. nidulans* cells were harvested at logarithmic phase (the approximate wet weight of harvested cells was 17 g), then washed once and suspended in 25 ml of buffer A (50 mM Mes [pH 6.5], 1 mM benzamidine, 1 mM ε-aminocaproic acid, 1 mM phenylmethylsulfonyl fluoride). The suspended cells were broken by two passages through a French pressure cell (Aminco, 20,000 p.s.i.) at 0 to 4°C. Unbroken cells and large fragments of cell debris were removed by low speed centrifugation (6,000*g*, 10 min). Membranes were pelleted from the supernatant by high speed centrifugation (110,000*g*, 1 h), then suspended in buffer A containing sodium pyrophosphate (10 mM) and sodium bromide (1 M) to 40 ml. After incubation

[1] This work was supported by a grant GM21827 from the National Institutes of Health, grant FG02-86ER13516 from the Department of Energy, and by University of Missouri Institutional Biomedical Research Grant RR 07053 from the National Institutes of Health. H. C. R. was a National Institutes of Health Predoctoral Trainee (DHHS 5 T32 GM07494).

[2] Present address: Biological Laboratory, Faculty of Education, Kumamoto University, Kumamoto 860, Japan.

on ice for 40 min, the membranes were collected by high speed centrifugation (as above), then washed once in buffer A (using the same suspension and centrifugation steps), and finally were suspended in about 15 ml buffer A to yield a Chl concentration of 0.6 mg/ml. DβD-maltoside[3] (Calbiochem-Behring) was added to the membrane suspension at a detergent to Chl ratio of 10:1 (w/w). After incubation on ice for 30 min, the preparation was centrifuged (110,000*g*, 1 h) and the supernatant, termed the maltoside extract, was collected. The maltoside treatment solubilized approximately 25 to 35% of the membrane Chl. Octylglucoside (Calbiochem) was then added to the maltoside extract to yield an octylglucoside to Chl ratio of 60:1 (w/w). The sample was incubated on ice for 30 min, then loaded onto linear sucrose density gradients (20 ml, 8–20% sucrose w/w; 2.5 ml of sample was loaded per gradient) containing buffer A and 0.03% (w/v) DβD-maltoside, and centrifuged for 3 h at 130,000*g* in a Ti 60 rotor. Two green bands (bands II and III) were in the middle and in the lower portion of the gradients, respectively. Material from a yellow band in the upper portion of the gradients (band I) was pooled and diluted with approximately four volumes of buffer A containing 0.03% (w/v) DβD-maltoside, was collected by centrifugation (130,000*g*, 12 h), and then resuspended in a minimal volume of 50 mM Pipes (pH 7.5) containing 0.03% (w/v) DβD-maltoside. This sample was passed through a DEAE-Sephacel column equilibrated in the suspension buffer. Almost all yellow material eluted in the void volume. The yellow eluate was applied to an Affi-Gel 501 organomercury column (Bio-Rad Laboratories) equilibrated in the same buffer. The yellow eluate in the void volume from the Affi-Gel column was centrifuged at 130,000*g* for 24 h in the Ti 60 rotor, and the upper clear portion of the centrifuged solution was discarded. The yellow fraction, containing the carotenoid-associated protein, was collected and resuspended in a small volume of 50 mM Pipes (pH 7.5) containing 0.03% (w/v) DβD-maltoside. The properties of the carotenoid on the columns and in ultracentrifugation suggest that the pigment was specifically bound to the protein.

Temperature-mediated phase partitioning of thylakoids was performed as described previously (4). The membranes used for these experiments were not washed with sodium pyrophosphate and sodium bromide solution.

Cytoplasmic membrane enriched fractions were obtained according to the method of Omata and Murata (24).

Measurements of Absorption and Fluorescence Spectra. Absorption spectra were measured with a Beckman DU-7HS spectrophotometer at 25°C at a scanning speed of 600 nm/min. Temperature-difference spectra of yellow fractions suspended in 50 mM Pipes (pH 7.5), 0.03% (w/v) DβD-maltoside, 50% (v/v) glycerol were generated by subtracting a spectrum obtained at 25°C from a 0°C spectrum. Low temperature (77 K) fluorescence emission spectra were measured with an SLM 8000 spectrofluorometer (SLM Instruments Inc.).

Other Measurements. PAGE in the presence of LDS was performed essentially as described in (12, 26). Samples for gel electrophoresis were solubilized at 0 or 70°C for 10 min. The marker proteins used as standards were BSA (67 kD), ovalbumin (45 kD), chymotrypsinogen A (25 kD), and Cyt *c* (12 kD). Proteins were detected by staining the gels with silver (35). Chl, phycocyanin, carotenoid, and protein concentrations were estimated as described previously (1, 2, 20, 21).

Immunochemical Detection of the 42 kD Polypeptide. Antibodies were raised against a 42 kD polypeptide according to the method of Chua and Blomberg (7), and Western blots were performed by the procedure of Towbin *et al.* (33). Antibody against the spinach 33 kD protein (the extrinsic protein of oxygen-evolving enzyme) was a gift from Dr. T. Kuwabara.

In some cases, antibody preparations were purified prior to use by a modification of the procedure described in Smith and Fisher (30). A λgt11 clone selected by screening an *A. nidulans* R2 genomic library (prepared by KJ Reddy and C Vann) with the total antibody raised against the 42 kD polypeptide was propagated in *Escherichia coli* Y1089 as a lysogen, and induced with IPTG (Bethesda Research Laboratories) at late-log phase to produce the antibody-reactive fusion protein (15). Total cellular protein was isolated from the lysogenized strain and fractionated according to Russel and Model (28); the antibody-reactive fusion polypeptide was found to be associated primarily with the membrane fraction of the cells. After electrophoresis of this fraction (LDS-PAGE on a 5–15% slab gel for 3 h at 2 W), the separated high mol wt components were transferred to nitrocellulose (33). The nitrocellulose filter was incubated with the total antibody preparation and developed as described in Smith and Fisher (30), except that o-dianisidine was used as the indicator dye. Antibodies reactive with a band migrating at the approximate mol wt of β-galactosidase were eluted from the nitrocellulose filter using several brief acidic (5 mM glycine, pH 2.3) rinses, and these washes were immediately neutralized by addition of pH 7.4 buffer (30). This solution (containing the purified antibodies) was used directly to probe nitrocellulose strips containing blotted *A. nidulans* R2 proteins, and ^{125}I-conjugated goat anti-rabbit antibody (New England Nuclear) was used to detect the primary antibody reaction.

RESULTS

Characteristics of Bands I and II Obtained from Sucrose Density Gradient. Yellow (band I) and green (bands II and III) bands were obtained from the sucrose density gradient. The red-region Chl absorption peak in normal *Anacystis nidulans* thylakoids is 680 nm; the absorption peak in band II was 676 nm, whereas band I, where the content of Chl was considerably reduced, had a Chl absorption peak at 671 nm (Fig. 1A). Excitation of *A. nidulans* thylakoids with 435 nm light at 77 K yields fluorescence emission peaks at 685, 695, and 716 nm (3); the 685 and 695 nm emission peaks are thought to arise from PSII-associated pigments, whereas the 716 nm peak is from PSI-associated Chl. Low temperature fluorescence emission spectra from excited Chl in band II exhibited a large peak at 685 nm, a shoulder at 695 nm, and very little fluorescence at 716 nm, indicating a significant enrichment of PSII relative to PSI in this fraction. By contrast, 77 K fluorescence emission spectra of band I Chl had a large peak at 685 nm and a shoulder at 673 nm (data not shown). Most of the PSI-associated Chl present in maltoside extracts of thylakoids migrated with band III in the sucrose density gradient, and was not characterized further.

The major polypeptides present in band I and in band II are shown in Fig. 1B. The total maltoside extract material is enriched in PSII-associated Chl-proteins at 52 and 45 kD (26). In band II material, these same two polypeptides are enriched still further, as is a 35 and a 42 kD component (Fig. 1B). A more detailed analysis of the PSII-enriched band II material will be the subject of a separate communication. Band I is depleted of the 52 kD PSII Chl-protein, but is slightly enriched in the 45 kD Chl-protein and in the 71 kD phycobilisome anchor component; the most dramatic enrichment in band I, however, is in a 42 kD polypeptide (Fig. 1B, band I).

Carotenoid-Associated Protein Fraction. The yellow fraction obtained by passage of band I material through chromatography columns was highly enriched in both carotenoids (free of Chl) and in the 42 kD polypeptide (Fig. 2, A and B). The *Synechocystis* PCC6714 carotenoprotein of cytoplasmic membranes changed electrophoretic mobility from 35 to 45 kD when heated (6); an

[3] Abbreviations: DβD-maltoside, dodecyl-β-D-maltoside; octylglucoside, octyl-β-D-glucopryanoside; LDS, lithium dodecyl sulfate; IPTG, isopropyl-β-D-thiogalactopyranoside.

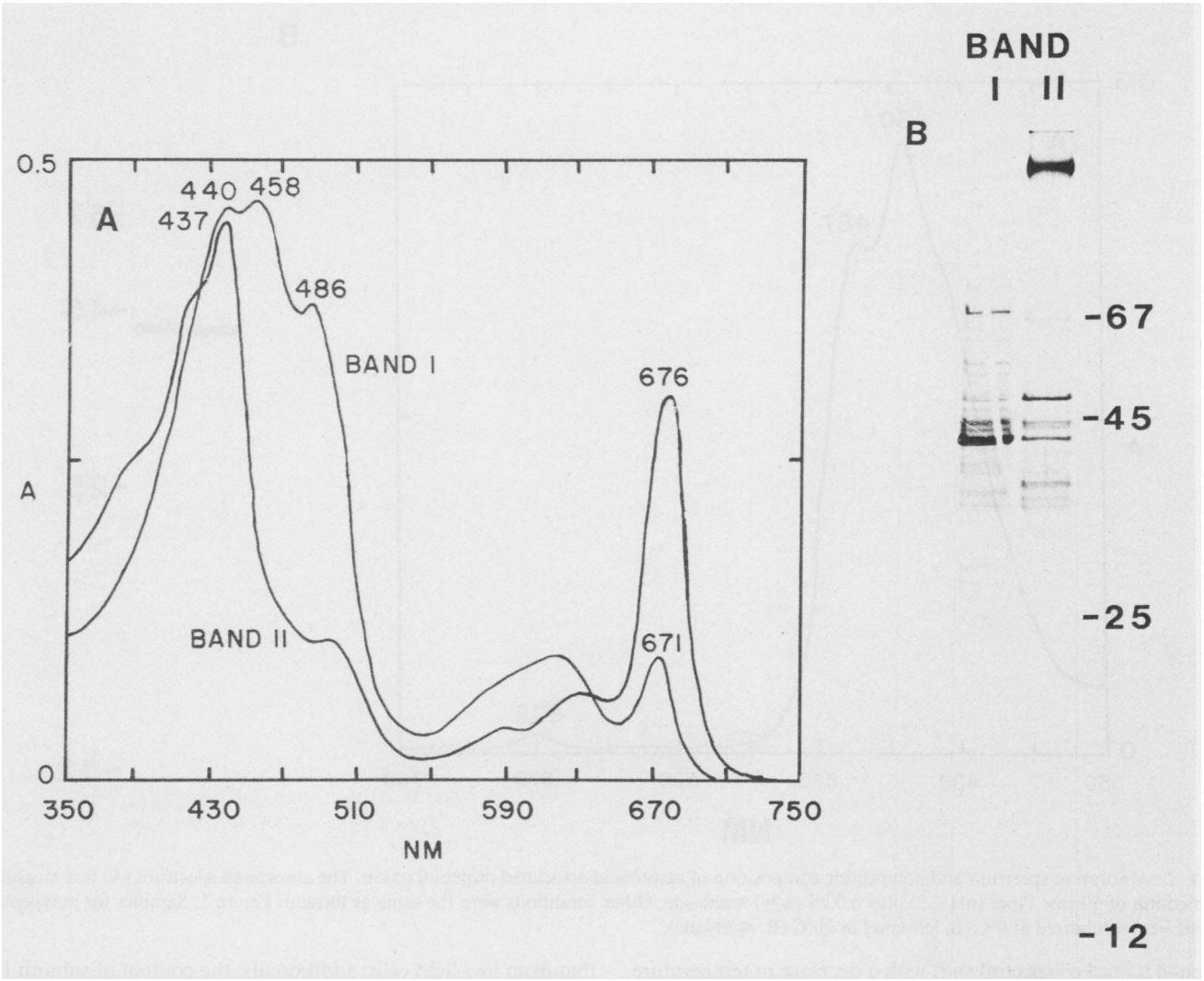

FIG. 1. Absorption spectra and polypeptide composition of bands I and II. Band I and band II were obtained by centrifugation of maltoside extracts on a sucrose density gradient. In A, the band preparations were suspended in buffer A prior to collection of absorption spectra at room temperature. B, polypeptide composition of band preparations (bands I and II). Polypeptides were analyzed by LDS-PAGE (13–18% acrylamide gradient) at 4°C, after solubilizing the samples of 0°C. The gels were stained with silver. The numerals at the right side in B show positions and mol wt (in kD) of marker proteins.

immunologically related component displaying the same electrophoretic behavior exists in *A. nidulans* R2 (5). By contrast, the 42 kD polypeptide had the same mobility upon electrophoresis whether solubilized at 0°C or at 70°C (Fig. 2B). A trace amount of a 45 kD polypeptide is detectable in this yellow fraction upon solubilization at 70°C; however, on the basis of the relative enrichment of both the 42 kD polypeptide and carotenoids during the purification procedure, this contaminant is not likely to be responsible for the binding of a significant amount of carotenoid present in the purified fraction.

Temperature-mediated phase partitioning with Triton X-114 can separate the intrinsic membrane proteins from extrinsic components (4). Western blot experiments with the separated fractions of *A. nidulans* membranes obtained by Triton X-114 phase partitioning showed that only the intrinsic membrane protein phase contained the 42 kD polypeptide (data not shown). Hence, this carotenoid-containing protein appears to be different from the aqueous-phase carotenoprotein reported in Holt and Krogmann (14).

Cytoplasmic membrane preparations from *A. nidulans* R2 are enriched in a carotenoid species which absorbs light at 390 nm; this absorption maximum is much reduced in purified thylakoid membranes (24). The presence of such an absorption maximum in the isolated carotenoprotein would strongly suggest a cytoplasmic membrane origin for this species. The absorption spectrum of the isolated carotenoid-associated protein fraction is shown in Figure 2A. The major absorption peaks are at 460 and 487 nm, with a shoulder at 435 nm, and only a trace amount of Chl can be detected. Thus, this isolated carotenoprotein shows relatively little absorbance due to a carotenoid species having a peak at 390 nm. The isolated carotenoprotein and purified cytoplasmic membrane preparations both have a major absorbance peak at 456 to 460 nm; the absorbance ratio (A_{390}:A_{456}) is therefore useful in assessing a possible contribution of cytoplasmic membrane carotenoids in these biochemical preparations. The A_{390}:A_{456} ratios were 0.2 for this fraction, and 0.8 for cytoplasmic membranes isolated according to Omata and Murata (24). The relative absorption intensity at 390 nm in this preparation is therefore consistent with a thylakoid membrane origin of this carotenoprotein.

Omata and Murata (24) demonstrated that, in *A. nidulans*, cytoplasmic membrane-associated carotenoid absorption spectra

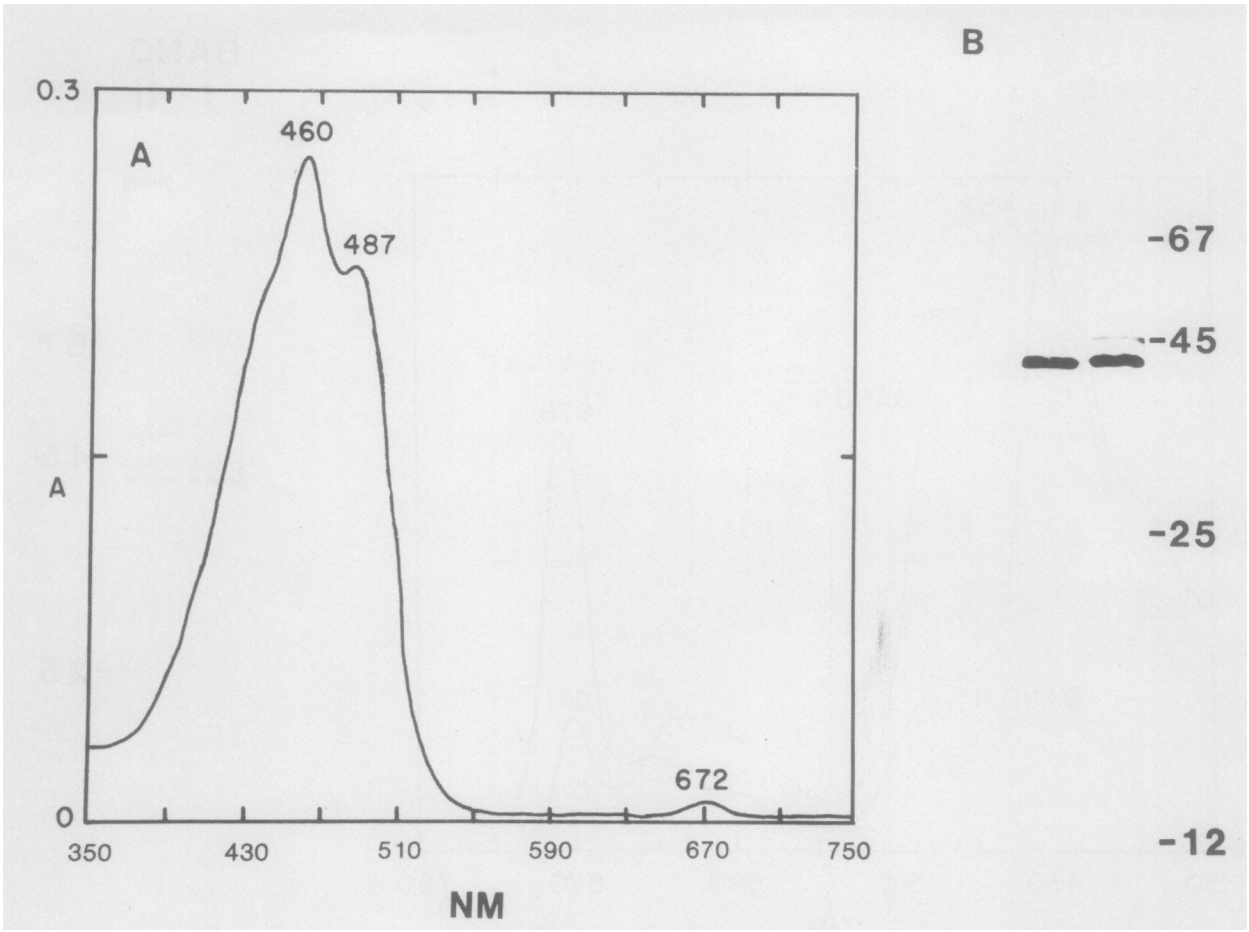

FIG. 2. Absorption spectrum and polypeptide composition of carotenoid-associated protein fraction. The absorption spectrum (A) was measured in a medium of 50 mM Pipes (pH 7.5) plus 0.03% (w/v) maltoside. Other conditions were the same as those in Figure 1. Samples for polypeptide analysis were solubilized at 0°C (B, left lane) or 70°C (B, right lane).

exhibited a marked spectral shift with a decrease in temperature, whereas thylakoid-associated carotenoids exhibited a much smaller shift. An absorbance-difference spectrum (0°C minus 28°C) of isolated cytoplasmic membranes shows a peak at 390 nm; the major carotenoid contributing to this peak seems to be zeaxanthin (11, 24). Cytoplasmic membrane enriched fractions obtained in our laboratory by the method of Omata and Murata (24) showed a similar spectrum (Fig. 3B). By contrast, our carotenoid fraction did not have such a peak in the difference spectrum (Fig. 3A), but yielded a reversible red-shift of carotenoid(s) (red-shift at 0°C). This result is similar to the difference spectrum obtained from the thylakoid fraction by Omata and Murata (24).

Accumulation of the 42 kD Polypeptide under High-Light Growth Conditions. *A. nidulans* cells grown under appropriate light conditions decrease their cellular content of PSI, whereas their phycobilisome and PSII content remain constant. This was shown to occur with cells grown in strong far red light (23), light primarily absorbed by Chl *a* (9). *A. nidulans* cells grown under high white light intensities responded in much the same way as cells grown in strong far-red light. Although the phycocyanin to Chl ratios of cells grown in high and low light were similar, a blue shift of 2 nm in the Chl absorption band was present in phycobilisome-depleted thylakoids from cells grown in high-light, relative to identically treated thylakoids from cells grown in low light (678 nm and 680 nm, respectively). The 716 nm peak of 77 K Chl fluorescence emission of phycobilisome-depleted thylakoids from high-light cells was much less intense than that from low-light cells; additionally, the content of subunit I of PSI (12) in membranes from high-light cells was significantly less than that in low-light cells (standardized to constant membrane protein, data not shown). Western blotting experiments indicated that the quantity of the 42 kD protein present in the carotenoid-containing fraction (described earlier) increased dramatically in cells grown under high light conditions (Fig. 4A). In addition, there was a simultaneous increase in carotenoid content (about 150% on a Chl basis) in cells grown under high light. As a control, we monitored the levels of the extrinsic polypeptide associated with the oxygen-evolving PSII complex. This protein, isolated originally from chloroplasts by Kuwabara and Murata (19), has a mol wt of 33 kD in chloroplasts, but migrates as a 29 kD band in *A. nidulans* R2. This protein was chosen to determine if levels of PSII proteins were affected by changes in light quantity; as shown in Fig. 4A, this protein remained relatively constant during growth at either light intensity. This suggests that the increase in the 42 kD polypeptide is a response to high light, and is not specifically coupled to cellular PSII content.

Iron-stressed *A. nidulans* R2 cells decrease their thylakoid membrane content to 25 to 30% of normally grown cells, and decrease substantially the relative quantity of membrane proteins involved in photosynthesis (on a constant membrane protein basis; 29; HC Riethman, LA Sherman, unpublished data). If the carotenoprotein was in fact thylakoid-associated and involved in protection of photosynthetic proteins from photooxidative damage, we would expect it to respond to iron stress in the same way as the other photosynthetically important thylakoid proteins.

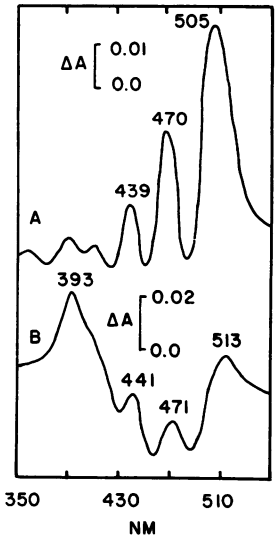

FIG. 3. Temperature-induced difference spectra (0°C minus 25°C) of the carotenoid-associated protein fraction and cytoplasmic membrane-enriched fraction. The cytoplasmic membrane-enriched fraction was obtained by the method of Omata and Murata (24). For measurement of the difference spectra, see "Materials and Methods."

Figure 4B is an immunoblot of electrophoretically separated components of *A. nidulans* membranes isolated from normal (+Fe) or iron-stressed (−Fe) cells. The 42 kD protein is detected only in normally grown cells, and is either absent or is much decreased in iron-stressed cells. This is consistent with both a photosynthesis-associated role and a thylakoid localization for this polypeptide.

The antibody generated against the purified carotenoprotein (Fig. 2B) was weakly reactive with several membrane components in addition to the strongly reactive 42 kD polypeptide, causing background bands to appear during immunostaining procedures (*e.g.* Fig. 4A, faint band at 35 kD). To eliminate background staining, we purified a fraction of the total antibody which was specific for a piece of the 42 kD polypeptide present on a β-galactosidase fusion protein ("Materials and Methods"). The purified antibody fraction was used to stain the immunoblot in Figure 4B. This purification of the antibody allowed us to confirm the identity of the 42 kD band as the major polypeptide stained in Figure 4A and, in addition, provides us with a highly specific reagent for further analysis of this protein.

DISCUSSION

A carotenoid-associated protein (42 kD) was isolated from DβD-maltoside extracts of *Anacystis nidulans* R2 membranes, and was enriched in the band I fraction from a sucrose density gradient (Fig. 1). This polypeptide is not a candidate for a cell wall-associated carotenoprotein for three reasons: (a) cell walls of *A. nidulans* have been reported to contain P750 (22), but band I had no such absorption peak (Fig. 1A); (b) the major polypeptides (50 and 52 kD) in the cell wall of *A. nidulans* (22, 27), are virtually absent in band I (Fig. 1B); and (c) the carotenoids of the cell wall fraction cannot be solubilized by Triton X-100 (18, 27), but the 42 kD polypeptide was easily solubilized by mild detergents.

The carotenoid composition of cytoplasmic membranes (where the major component is zeaxanthin) is known to be different from that in thylakoids (where the major carotenoid is β-carotene), and cytoplasmic membranes show a 390 nm peak in a temperature-induced difference spectra (11, 24). The isolated carotenoid-associated protein showed little contribution from

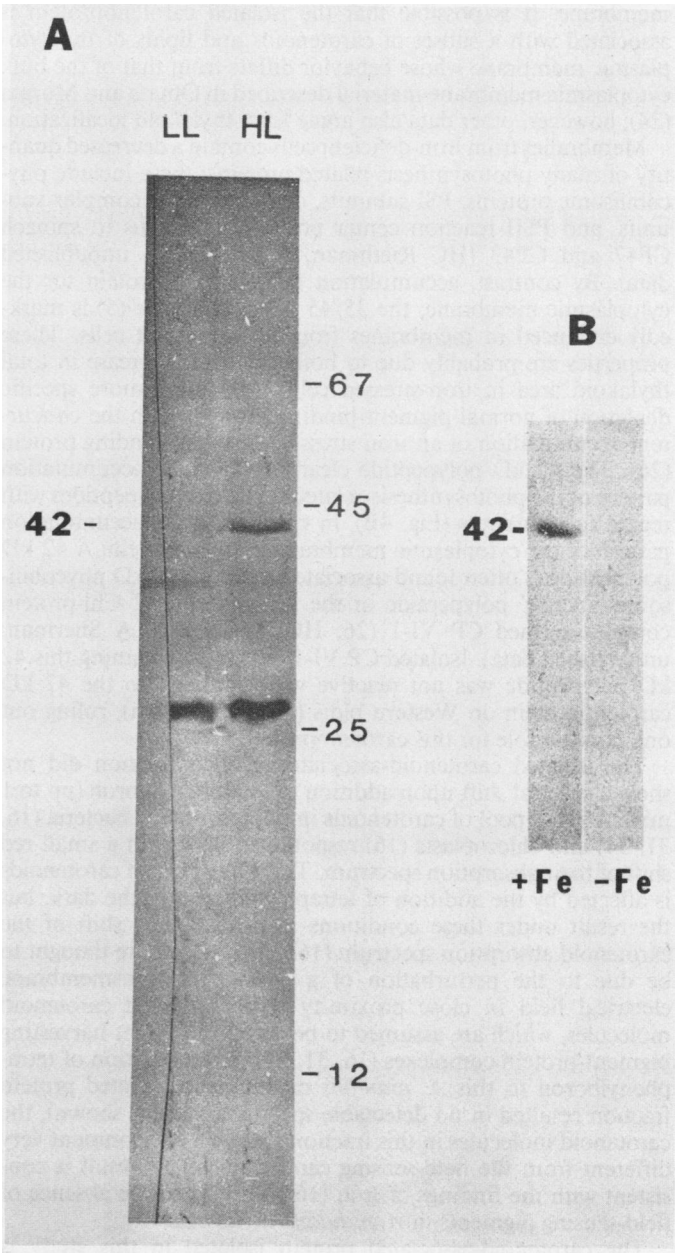

FIG. 4. Detection of immunoreactive components of membranes from cells grown under altered environmental conditions. Panel A, membranes isolated from cells grown in either low light (LL; 0.02 mW/cm^2) or high light (HL; 1.9 mW/cm^2) were fractionated using LDS-PAGE, and the separated components were electroblotted onto nitrocellulose filters. The left half of the LL lane was probed with antibody to the isolated 42 kD polypeptide, whereas the right half of the LL lane and and the entire HL lane were probed with a mixture of antibody against the 42 kD polypeptide and antibody against the extrinsic 33 kD protein from spinach chloroplast oxygen-evolving particles. The membranes were solubilized at 0°C. The loaded amount of protein was 30 μg per lane. Panel B, membranes isolated from normal (+Fe) and iron-deficient (−Fe) cells were electrophoresed and blotted as in panel A, then probed with the affinity-purified antibody fraction described in "Materials and Methods." Detection of the primary antibody was with ^{125}I-conjugated goat anti-rabbit antibody, followed by autoradiography of the nitrocellulose filters.

this 390 nm absorbing carotenoid (Figs. 2 and 3), suggesting a different carotenoid composition from that in the cytoplasmic membrane. It is possible that the isolated carotenoprotein is associated with a subset of carotenoids and lipids of the cytoplasmic membrane whose behavior differs from that of the bulk cytoplasmic membrane material described in Omata and Murata (24); however, other data also argue for a thylakoid localization.

Membranes from iron-deficient cells contain a decreased quantity of many photosynthesis-related proteins; these include phycobilisome proteins, PSI subunits, cytochrome b_6f complex subunits, and PSII reaction center proteins analogous to spinach CP47 and CP43 (HC Riethman, LA Sherman, unpublished data). By contrast, accumulation of a marker protein for the cytoplasmic membrane, the 35/45 kD polypeptide (5) is markedly enhanced in membranes from iron-deficient cells. These properties are probably due to both a general decrease in total thylakoid area in iron-stressed cells (29) and a more specific depletion of normal pigment-binding proteins with the concurrent accumulation of an iron stress-specific Chl-binding protein (26). The 42 kD polypeptide clearly follows the accumulation pattern of the photosynthesis-related thylakoid polypeptides with regard to iron stress (Fig. 4B), in contrast to the accumulation pattern of the cytoplasmic membrane marker protein. A 42 kD polypeptide is often found associated with the 71 kD phycobilisome 'anchor' polypeptide in the *A. nidulans* R2 Chl-protein complex termed CP VI-1 (26; HC Riethman, LA Sherman, unpublished data). Isolated CP VI-1 material containing this 42 kD polypeptide was not reactive with antibody to the 42 kD carotenoprotein on Western blots (data not shown), ruling out one possible role for this carotenoprotein.

The isolated carotenoid-associated protein fraction did not show a spectral shift upon addition of tetraphenylboron (up to 1 mM). A small pool of carotenoids in photosynthetic bacteria (16, 31, 34) and chloroplasts (16) responds to light with a small red shift of their absorption spectrum. This same pool of carotenoids is affected by the addition of tetraphenylboron in the dark, but the result under these conditions is a small blue shift of the carotenoid absorption spectrum (16). Both effects are thought to be due to the perturbation of a permanent transmembrane electrical field in close proximity to the affected carotenoid molecules, which are assumed to be attached to light-harvesting pigment-protein complexes (16, 31, 34). Since addition of tetraphenylboron to this *A. nidulans* carotenoid-associated protein fraction resulted in no detectable spectral shift (not shown), the carotenoid molecules in this fraction are in an environment very different from the field-sensing carotenoids. This result is consistent with the findings of Itoh (16), who noted the absence of field-sensing pigments in *A. nidulans* thylakoids.

The carotenoid-associated protein isolated in this study is clearly different from the species isolated from the cytoplasmic membrane as described previously (5, 6). We have not been able to separate cleanly the cytoplasmic membrane from thylakoids in this strain of *A. nidulans*; hence, we present more indirect evidence which leads us to conclude that this carotenoid-binding protein is thylakoid-associated. More importantly, we have clearly shown that this carotenoid-associated protein does not accumulate in cells grown under low light conditions (0.02 mW/cm^2), but becomes a fairly abundant membrane component in cells grown under both moderate (0.5 mW/cm^2) and high (1.9 mW/cm^2) light intensities. Although a specific role for this carotenoprotein has not yet been ascertained, the availability of the antibody will likely enable us to establish firmly the intracellular localization (through electron microscopy of immunogold-labeled cells) and possibly the *in vivo* function of this light-induced carotenoprotein (through selection, mutagenesis, and reintroduction of DNA clones encoding this protein).

Acknowledgments—The authors would like to thank Jill Cunningham for her excellent technical help, Dr. K. J. Reddy for the use of the λgt11 library, Dr. T. Kuwabara for the gift of antisera to the spinach 33 kD protein, and Dr. G. S. Bullerjahn for helpful discussions.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. ARNON DI, BD MCSWAIN, HY TSUJIMOTO, K WADA 1974 Photochemical activity and components of membrane preparations from blue-green algae. 1. Coexistence of two photosystems in relation to chlorophyll a and phycocyanin. Biochim Biophys Acta 357: 231–245
3. BRICKER TM, JA GUIKEMA, HB PAKRASI, LA SHERMAN 1986 Proteins of cyanobacterial thylakoids. *In* LA Staehelin, CJ Arntzen, eds, Encyclopedia of Plant Physiology, Vol 19. Springer-Verlag, Berlin, pp 640–652
4. BRICKER TM, LA SHERMAN 1984 Triton X-114 phase fractionation of membrane proteins of the cyanobacterium *Anacystis nidulans* R2. Arch Biochem Biophys 235: 204–211
5. BULLERJAHN GS, HC RIETHMAN, LA SHERMAN 1987 Isolation and immunological characterization of a carotenoid-binding protein from the cyanobacteria *Synechocystis* sp. strain PCC714 and *Anacystis nidulans* R2. *In* J Biggins, ed, Progress in Photosynthesis Research, Vol II. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp 145–148
6. BULLERJAHN GS, LA SHERMAN 1986 Identification of a carotenoid-binding protein in the cytoplasmic membrane from the heterotrophic cyanobacterium *Synechocystis* sp. strain PCC6714. J Bacteriol 167: 396–399
7. CHUA N-H, F BLOMBERG 1979 Immunochemical studies of thylakoid membrane proteins from spinach and *Chlamydomonas reinhardtii*. A modified procedure for crossed immunoelectrophoresis of dodecyl sulfate-protein complexes. J Biol Chem 254: 215–223
8. DREWS G, J WECKESSER 1982 Function, structure, and composition of cell walls and external layers. *In* NG Carr, BA Whitton, eds, The Biology of Cyanobacteria. Blackwell Scientific Publications Ltd, Oxford, pp 333–358
9. FUJITA Y, K OHKI, A MURAKAMI 1985 Chromatic regulation of photosystem composition in the photosynthetic systems of red and blue-green algae. Plant Cell Physiol 26: 1541–1548
10. GOLECKI JR, G DREWS 1982 Supramolecular organization and composition of membranes. *In* NG Carr, BA Whitton, eds, The Biology of Cyanobacteria. Blackwell Scientific Publications Ltd, Oxford, pp 125–141
11. GOMBOS Z, L VIGH 1986 Primary role of the cytoplasmic membrane in thermal acclimation evidenced in nitrate-starved cells of the blue-green alga, *Anacystis nidulans*. Plant Physiol 80: 415–419
12. GUIKEMA JA, LA SHERMAN 1982 Protein composition and architecture of the photosynthetic membranes from the cyanobacterium, *Anacystis nidulans* R2. Biochim Biophys Acta 681: 440–450
13. GUIKEMA JA, LA SHERMAN 1984 Influence of iron deprivation on the membrane composition of *Anacystis nidulans*. Plant Physiol 74: 90–95
14. HOLT TK, DW KROGMANN 1981 A carotenoid-protein from cyanobacteria. Biochim Biophys Acta 637: 408–414
15. HUYNH TV, RA YOUNG, RW DAVIS Constructing and screening cDNA libraries in λgt11. *In* DM Glover, ed, DNA Cloning, Vol 1. IRL Press, Washington DC, pp 49–78
16. ITOH S 1984 Absorbance changes of carotenoids and bacteriochlorophylls responding to the change of local electrical field induced by hydrophobic anion, tetraphenylborate in membranes of photosynthetic bacteria. Biochim Biophys Acta 766: 464–471
17. JURGENS UJ, J WECKESSER 1985 Carotenoid-containing outer membrane of *Synechocystis* sp. strain PCC6714. J Bacteriol 164: 384–389
18. JURGENS UJ, J WECKESSER 1985 Characterization of the cell wall of the unicellular cyanobacterium *Synechocystis* PCC6714. Arch Microbiol 142: 168–174
19. KUWABARA T, N MURATA 1979 Purification and characterization of 33 kilodalton protein of spinach chloroplasts. Biochim Biophys Acta 581: 228–236
20. LIAAEN-JENSEN S, A JENSEN 1971 Quantitative determination of carotenoids in photosynthetic tissues. Methods Enzymol 23: 586–602
21. MARKWELL MAK, SM HASS, LL BIEBER, NE TOLBERT 1978 A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87:206–210
22. MURATA N, N SATO, T OMATA, T KUWABARA 1981 Separation and characterization of thylakoid and cell evelope of the blue-green alga (cyanobacterium) *Anacystis nidulans*. Plant Cell Physiol 22:855–866
23. MYERS J, J-R GRAHAM, RT WANG 1980 Light harvesting in *Anacystis nidulans* studied in pigment mutants. Plant Physiol 66: 1144–1149
24. OMATA T, N MURATA 1983 Isolation and characterization of the cytoplasmic membranes from the blue-green alga (cyanobacterium) *Anacystis nidulans*. Plant Cell Physiol 24: 1101–1112
25. OMATA T, N MURATA 1984 Isolation and characterization of three types of membranes from the cyanobacterium (blue-green alga) *Synechocystis* PCC6714. Arch Microbiol 139: 113–116
26. PAKRASI HB, HC RIETHMAN, LA SHERMAN 1985 Organization of pigment proteins in the photosystem II complex of the cyanobacterium *Anacystis nidulans* R2. Proc Natl Acad Sci USA 82: 6903–6907
27. RESCH CM, J GIBSON 1983 Isolation of the carotenoid-containing cell wall of

three unicellular cyanobacteria. J Bacteriol 155: 345–350
28. RUSSEL M, P MODEL 1982 Filamentous phage pre-coat is an integral membrane protein: analysis by a new method of membrane preparation. Cell 28: 177–184
29. SHERMAN DM, LA SHERMAN 1983 Effects of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*. J Bacteriol 156: 393–401
30. SMITH DE, PA FISHER 1984 Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J Cell Biol 99: 20–28
31. SYMONS M, C SWYAEN, C SYBESMA 1977 The light-induced absorbance change in *Rhodopseudomonas sphaeroides*. Biochim Biophys Acta 462: 706–717
32. THORNBER JP 1986 Biochemical characterization and structure of pigment-proteins of photosynthetic organism. *In* LA Staehelin, CJ Arntzen, eds, Encyclopedia of Plant Physiology, vol 19. Springer-Verlag, Berlin, pp 98–142
33. TOWBIN H, T STAEHELIN, J GORDON 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350–4354
34. WRAIGHT CA, RJ COGDELL, B CHANCE 1978 Ion transport and electrochemical gradients in photosynthetic bacteria. *In* RK Clayton, WR Sistrom, eds, The Photosynthetic Bacteria. Plenum Press, New York, pp 471–511
35. WRAY W, T BOULIKAS, VP WRAY, R HANCOCK 1981 Silver staining of proteins in polyacrylamide gels. Anal Biochem 118: 197–203