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Isolation and Characterization of a Protein Associated with Carotene Globules in the Alga *Dunaliella bardawil*[1]

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The halotolerant alga *Dunaliella bardawil* accumulates very large amounts of β-carotene when exposed to high light intensity. The accumulated β-carotene is concentrated in small, oily globules within the chloroplast and has been suggested to protect the alga against photodamage by high irradiation (A. Ben-Amotz, A. Katz, M. Avron [1982] J Phycol 18: 529–537; A. Ben-Amotz, M. Avron [1983] Plant Physiol 72: 593–597; A. Ben-Amotz, A. Shaish, M. Avron [1989] Plant Physiol 91: 1040–1043). A 38-kD protein was identified and purified from β-carotene globules and was designated carotene globule protein (Cgp). Induction of Cgp occurs in parallel with β-carotene accumulation in *D. bardawil* grown under different inductive conditions. Cgp is overproduced in a constitutive mutant strain that overproduces β-carotene and is not detected in *Dunaliella salina*, a species that does not accumulate β-carotene. Cgp production was not suppressed by norflurazon, an inhibitor of β-carotene synthesis that leads to accumulation of the carotenoid precursor phytoene. Immunogold-labeling analysis by electron microscopy demonstrates that the protein is localized at the periphery of the globules. Proteolytic cleavage by trypsin enhances the coalescence and destruction of the globules, in parallel with Cgp disappearance. It is suggested that the function of Cgp is to stabilize the structure of the globules within the chloroplast.

The halotolerant green alga *Dunaliella bardawil* accumulates massive amounts of β-carotene when exposed to HL intensities, nutrient deprivation, and other stress conditions. It has been demonstrated that cells containing large amounts of β-carotene-rich globules are resistant to photodamage at very high light intensities, suggesting that β-carotene protects the cells from photoinhibition (Ben-Amotz et al., 1987, 1989). The massive amount of β-carotene is organized in minute lipid globules of 100 to 200 nm that are located in the interthylakoid space of the chloroplast (Ben-Amotz et al., 1982). The small diameter of these globules is probably a critical factor in obtaining homogeneous distribution without disrupting the delicate chloroplast structure. Isolated globules are stable in aqueous solutions, suggesting that they possess a stabilizing layer that prevents their aggregation and coalescence. However, there is no information concerning the elements stabilizing these β-carotene globules in *Dunaliella*.

Different types of plant lipid bodies covered by a stabilizing layer have been described. Oil bodies in seeds, which contain primarily triacylglycerols, are enclosed by a monolayer of phospholipids and special low-molecular-weight proteins, termed oleosins (Huang, 1992; Tzen and Huang, 1992). Chromoplast lipoprotein fibrils, which contain carotenoids, are enclosed by polar lipids and by a protein termed fibrillin (Deruére et al., 1994). Another protein that is co-induced and specifically associated with carotenoid bodies within chromoplasts was identified in corollas of *Cucumis sativum* flowers (Smirra et al., 1993).

Earlier studies in our laboratory showed that β-carotene globules isolated from *D. bardawil* contain exclusively β-carotene, neutral lipids, and a small amount of protein. However, the proteins have not been characterized and their function is not known (Ben-Amotz et al., 1982).

In this paper we report the isolation and purification of a protein from β-carotene globules designated Cgp. We demonstrate that Cgp is co-induced with β-carotene under a variety of conditions and show that it stabilizes the globules and prevents their coalescence.

MATERIALS AND METHODS

Algae and Growth Conditions

Dunaliella bardawil wild-type Ben-Amotz and Avron, a local isolated species, is deposited in the American Type Culture Collection (Rockville, MD, No. 30861). The mutant Db-8 is a β-carotene-overproducing mutant of *D. bardawil* developed by Dr. Aviv Shaish (Shaish et al., 1991). *Dunaliella salina* was obtained from the culture collection of Dr. W.H. Thomas (La Jolla, CA). This *D. salina* species is unable to accumulate β-carotene. The algae were grown in a medium containing 2 M NaCl under continuous light and shaking as described previously (Ben-Amotz et al., 1989).

Three different light intensity regimes were used: LL, a growth incubator illuminated with cool-white fluorescent lamps providing light at 10 W/m^2; HL, a Warburg apparatus equipped with halogen lamps providing light at 500 W/m^2; and outdoor cultures with natural illumination.

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Abbreviations: Cgp, carotene globule protein; HL, high light; LL, low light; octyl-POE, *n*-octyl-pentaoxyethylene; SBTI, soybean trypsin inhibitor.

Nitrate Starvation

Algae were washed twice in a nitrate-free growth medium and incubated under LL intensity for 2 to 3 d to induce β-carotene production (Shaish et al., 1990).

Norflurazon Treatment

Norflurazon (3.0×10^{-7} M) was added to algae that were induced for β-carotene synthesis by starvation to nitrate (Shaish et al., 1990).

Globule Isolation Procedure

Isolation of globules was performed as previously described (Jiménez and Pick, 1994). Algae were ruptured osmotically and then centrifuged at low speed for collection of chloroplasts. The chloroplasts were disrupted by Yeda press (Limca, Tel Aviv, Israel) and the thylakoid membranes were separated from the supernatant containing globules by a low-speed centrifugation. Purification of the globules was performed by flotation on a discontinuous Suc gradient consisting of three layers (30% Suc containing the globule fraction; 5% Suc, 10 mM Tris-HCl, pH 8) and centrifugation at 75,000*g* for 2 h. The crude globules, recovered from the top fraction, were collected and repurified by flotation on a second Suc gradient. The purified globules were collected from the top and kept frozen in liquid nitrogen.

Cgp Extraction and Purification

Proteins were extracted from the globules by solubilization with detergent as follows. A final concentration of 0.3% to 0.5% Triton X-100 or octyl-POE (C_8E_5, Bachem, Torrance, CA) was added to 0.2 mL of globule suspension and incubated on ice for 15 min. The proteins were separated from the globules on a step Suc gradient consisting of 0.3 mL of 15% Suc, containing the detergent-treated globules, 3 mL of 5% Suc, and 1 mL of 0% Suc, all containing 10 mM Tris-HCl. The gradient was centrifuged at 75,000*g* for 2 h. The bottom fraction contained the solubilized proteins, and the top fraction contained the protein-depleted globules.

The extracted proteins were applied to a 30- × 1.5-cm Sephadex G-150 column equilibrated with 10 mM Tris-HCl, pH 7.5, 10% glycerol, 0.4% octyl-POE. Elution was carried out with the same buffer, and 1-mL fractions were collected. The fractions were analyzed for β-carotene content by a Bausch and Lomb (Rochester, NY) Spectronics 1201 spectrophotometer at 480 nm. The proteins of each fraction were precipitated and separated on SDS-PAGE as described below.

Generation of Anti-Cgp Antibodies

Antibodies were raised in rabbits as follows. Purified protein was resolved on 12% acrylamide SDS-PAGE, the gel was stained with 0.1% Coomassie blue in H_2O, and the 38-kD polypeptide was excised and washed three times with PBS. The gel slices were broken by passage through a series of syringes and mixed with an equal volume of complete Freund's adjuvant before 100 to 150 μg of protein were injected into each rabbit. The serum was collected after two boosts (21 d after the first immunization and 14 d after first boost).

Preparation of Samples for Protein Analysis

Culture samples containing 2.5×10^6 cells were centrifuged at 2000*g* for 10 min, and the pellet was immediately resuspended in 0.1 mL of H_2O, followed by the addition of 50 μL of 3× loading buffer (Laemmli, 1970) and incubation at 90°C for 2 min. Globules, protein extracts, or purified fractions were precipitated overnight by 80% acetone at −20°C. The pellet was resuspended in loading buffer without reducing agent and dye. After the protein content was assayed by the bicinchoninic acid method (Smith et al., 1985), the reducing agent and the dye were added and incubated for 2 min at 90°C.

Gel Electrophoresis and Immunoblot Analysis

Cell extracts or protein fraction extracts were analyzed by SDS-PAGE (Laemmli, 1970), 10 or 12% acrylamide as indicated. Gels were stained with 0.25% Coomassie blue in 50% methanol and 10% acetic acid or immunoblotted with anti-Cgp antibodies. Proteins were transferred to nitrocellulose (Towbin et al., 1979), and then the blot was incubated overnight at 4°C with PBS, 0.05% Tween, and 10% low-fat milk (T-PBS milk buffer). The blot was incubated for 1 h at room temperature with anti-Cgp antibodies at a dilution of 1:2000 in T-PBS buffer and washed extensively with T-PBS milk buffer. The blot was incubated at room temperature for 1 h with anti-rabbit IgG peroxidase conjugate diluted 1:1500 in T-PBS and then washed in the same buffer. Bound antibodies were detected with the ECL (enhanced chemiluminescence) detection system (Amersham).

Amino Acid Sequence

Proteolysis of the 38-kD band and the lower molecular mass bands was performed according to the method of Cleveland et al. (1977). Purified protein fractions of the polypeptides were applied to 12% acrylamide SDS-PAGE, and each protein band was excised from the stained gel and applied to a second SDS gel (15% acrylamide) in the presence of *Staphylococcus aureus* (V8) protease (2 μg/lane). The digestion proceeded for 30 min directly in the stacking gel. The peptide fragments were separated by electrophoresis and blotted on polyvinylidene difluoride membrane (Matsudaira, 1987). The N-terminal amino acid sequence of two major fragments was analyzed on Applied Biosystems' model 475A automatic pulsed liquid-gas phase protein microsequencer equipped with a model 120A online HPLC phenylthiohydantoin amino acid analyzer and a model 900A data acquisition and processing unit.

EM and Immunolabeling

Purified globules were treated with 10 μg/mL trypsin for 3 min, followed by 40 μg of SBTI to stop the reaction,

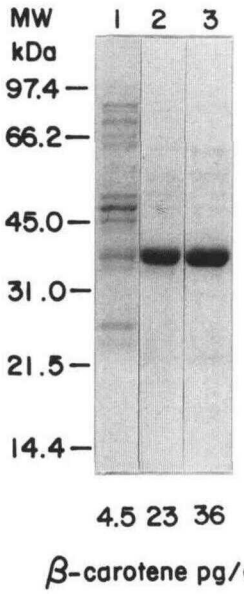

Figure 1. Protein analysis of purified globules. Coomassie blue-stained SDS-PAGE gel (10% acrylamide) of proteins extracted from globules derived from algae grown under different conditions. Lane 1, Globules prepared from wild-type cells grown under LL, noninducing conditions. Lane 2, Globules prepared from wild-type cells grown under HL, inducing conditions. Lane 3, Globules prepared from Db-8 mutant algae grown under LL. The amount of β-carotene accumulated in the cells is shown under each lane. Each lane contained 25 μg of protein.

and immediately applied to a grid, dried, stained with 2% uranyl acetate, and examined in a Philips (Eindhoven, The Netherlands) model 410 transmission electron microscope operated at 80 kV. For immunolabeling, cells were fixed, cryosectioned, and labeled as previously described (Sadka et al., 1991). A negative contrast stain was used as described by Himmelhoch (1994).

Light-Scattering Measurements

A suspension of 2 mL of purified globules in 10 mM Tris-HCl, pH 7.5, containing 20 μg of carotene, was placed in a cuvette in the SLM 8000 spectrofluorimeter (SLM-Aminco, Urbana, IL). Light-scattering changes were measured at 90°; the emission and excitation wavelengths were set at 350 nm. *A* changes were monitored online. Trypsin (10 μg), SBTI (40 μg), and detergent octyl-POE (0.5%) were added directly to the cuvette at the indicated times.

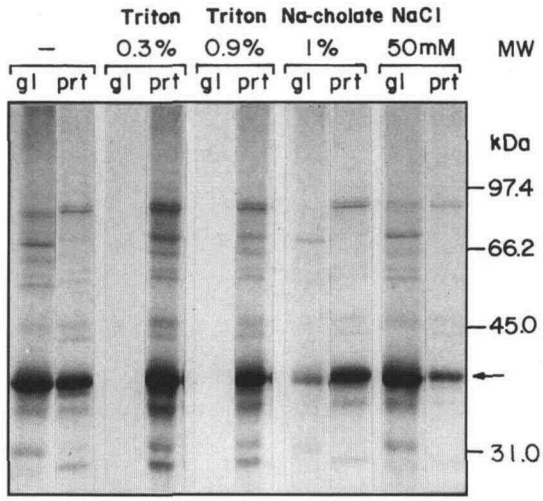

Figure 2. Detergent extraction of globule proteins. Globules isolated from the Db-8 mutant, equivalent to 350 μg of β-carotene, were treated with the indicated detergents or with 50 mM NaCl and separated by flotation on discontinuous Suc gradients. SDS-PAGE (10% acrylamide) analysis of the proteins of the total upper-phase globule fraction (gl) and the lower-phase-extracted protein fraction (prt) are shown. The arrow indicates Cgp location.

Analytical Procedures

Cell number was determined in a Coulter Counter model ZM (Coulter Electronics Ltd., Luton, UK) with a 100-μm aperture. β-Carotene was extracted from the algal pellet with acetone, diluted with H_2O to 80%, and assayed as previously described (Ben-Amotz and Avron, 1983). *A* spectra were monitored by a Bausch and Lomb Spectronics 1201 computerized spectrophotometer.

HPLC analysis was performed as previously described (Jiménez and Pick, 1994). β-Carotene was extracted from globules with ethanol:hexane (2:1). Pigments were analyzed by separation on a C_{18} reversed-phase HPLC column (VydacTP [Hesperia, CA] 20/154 stainless steel column of 25 cm × 4.6 mm, 5-μm particle size) and coupled to a Waters system equipped with a photodiode array detector. Elution was performed isocratically with methanol at 1 mL/min.

RESULTS

Protein Components of β-Carotene Globules

Previously we developed a procedure for isolation of β-carotene globules from *D. bardawil* cells (Ben-Amotz et

Table I. *β-Carotene and protein content of fractions during globule isolation*

Globules were isolated from Db-8 mutant algae grown under HL. Protein and β-carotene amounts were analyzed in each fraction as described in "Materials and Methods." β-Carotene isomer compositions were calculated from the HPLC peak areas. Car, β-Carotene.

Fraction	Protein	Carotene	Car/Protein	Car Isomers (9-*cis*/all-*trans*)
	mg	*mg*	*w/w*	
Chloroplast	140.0	109.0	0.7	1.8
Crude globules	18.0	52.0	2.8	1.8
Purified globules	9.5	38.0	4.0	2.0

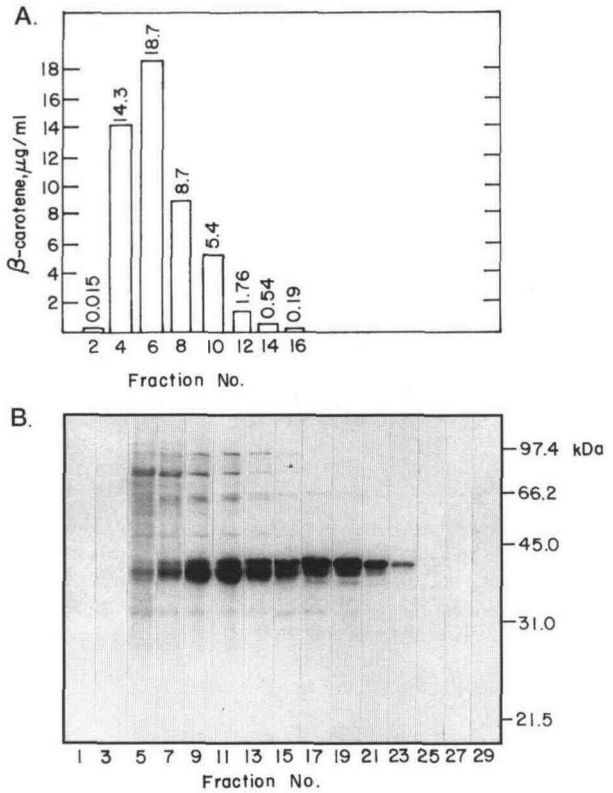

Figure 3. Partial resolution of detergent-extracted globule proteins and β-carotene on Sephadex G-150. Purification of proteins extracted from globules isolated from Db-8 mutant cells grown under HL. A, β-Carotene content of fractions eluted from the column. B, Analysis of the fractions by SDS-PAGE (12% acrylamide). Each lane contains an equal fraction volume.

al., 1982; Jiménez and Pick, 1994). EM and pigment analysis of the purified globules showed that they were essentially free of Chl or contaminating membranes and contained practically only neutral lipids, most of which were β-carotene, and a small amount of protein that has not been characterized. For protein composition analysis, β-carotene globules were extracted with acetone as described in "Materials and Methods" and separated on SDS-PAGE (Fig. 1). Globules isolated from HL-grown cells were highly enriched in a major polypeptide of about 38 kD (lane 2), which was hardly evident in the same fraction isolated from LL-grown cells, which did not accumulate β-carotene (lane 1). Conversely, the 38-kD polypeptide was enriched in a constitutive mutant strain, Db-8, which overproduced β-carotene under noninductive conditions (lane 3).

The overall yield of β-carotene and protein during the isolation procedure of globules derived from the Db-8 mutant is shown in Table I. The ratio of β-carotene to protein increased throughout the isolation and was 4:1 (w/w) in the purified globule fraction. The β-carotene in the purified globules was composed of the two characteristic isomers, 9-*cis* and all-*trans*, and the ratio between them remained constant throughout the isolation.

Isolation and Purification of Cgp, the Protein Associated *with Globules in Vivo*

The proteins were resolved from the globules by treatment with detergents, Triton X-100 or Na-cholate, followed by separation on a stepwise Suc gradient (see "Materials and Methods"). Under these conditions most (90%) of the carotene floated to the upper buffer layer, whereas essentially all of the protein remained in the lower Suc phase, indicating that it was dissociated from the globules (Fig. 2). It should be noted that the protein fraction still contained residual amounts of β-carotene, which was further resolved as described below. Significant dissociation of the protein occurred also in the absence of detergent (left two lanes); however, addition of 50 mM NaCl considerably decreased this dissociation (right two lanes).

Further purification of Cgp, obtained from the Db-8 mutant globules, was achieved by gel filtration of detergent-solubilized protein on a Sephadex G-150 column (Fig. 3). The purification separated Cgp from β-carotene (Fig. 3A) and from most contaminating proteins (Fig. 3B). It also partially resolved between two similarly sized polypeptides (Fig. 3B). The lower molecular mass polypeptide appeared only in globules extracted from the mutant strain (see following figures).

The purified 38-kD polypeptide was fragmented with protease V8, and the N-terminal amino acid sequence of

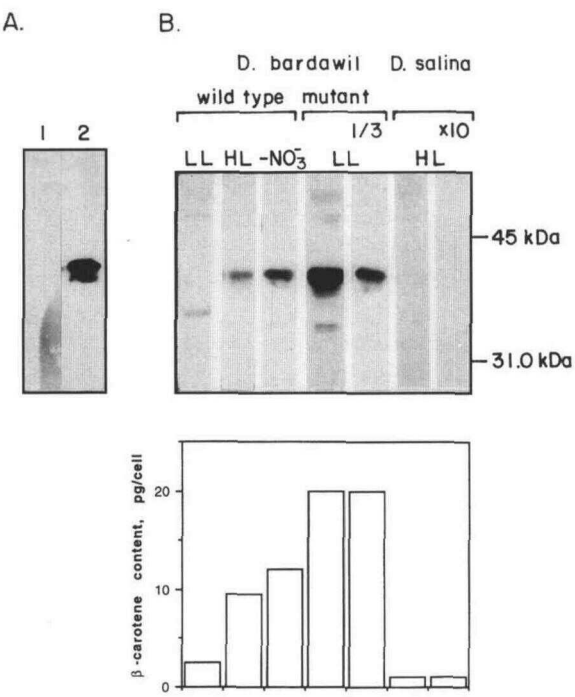

Figure 4. Immunoblot analysis with polyclonal anti-Cgp antibodies. A, Db-8 mutant globule protein extract. Lane 1, Preimmune serum; lane 2, immune serum. Each lane contains 10 μg of protein. B, Crude cell extracts of algae grown under various conditions as described in Figure 1. Each lane was loaded with 5×10^5 cells, except for the *D. bardawil* Db-8 mutant and *D. salina;* lane 1/3 was loaded with 1.6×10^5 cells, and lane x10 was loaded with 5×10^6 cells. The immune serum was diluted 1:2000-fold. The bottom panel represents the content of β-carotene in cells of each lane.

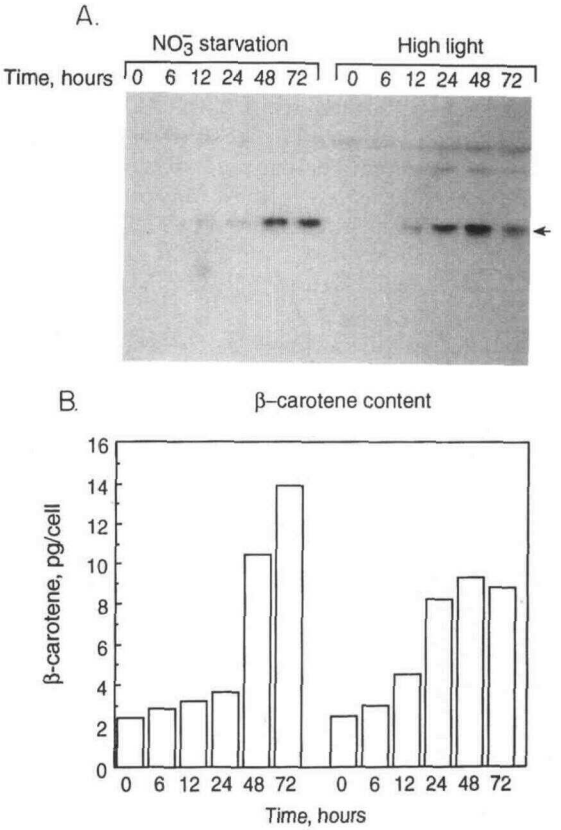

Figure 5. Induction kinetics of Cgp and β-carotene. A, Immunoblot analysis of extracted cells, with anti-Cgp antibodies, exposed to HL or nitrate starvation as described in "Materials and Methods." Time indicates the period of exposure to the induction conditions. B, Induction of β-carotene in the cells. The analysis was performed in wild-type *D. bardawil* cells.

fragments was determined as described in "Materials and Methods." The sequence of amino acid residues for one fragment of Cgp was established as LHDLRPCG-PYTAVMR. A comparison to DNA and protein sequence libraries did not reveal any meaningful similarities to this sequence.

Identification of Cgp with Antibodies

The 38-kD polypeptide of the purified protein fractions was excised from the gel and used to immunize rabbits. Figure 4A shows the specific cross-reaction of the immune serum with the 38-kD and lower molecular mass polypeptides in the globule protein extract from the Db-8 mutant. The antibodies were utilized to test the correlation between induction of Cgp synthesis and β-carotene accumulation. Total protein extracts of cells cultured under different conditions were probed with the antibodies (Fig. 4B), and the β-carotene content in cells was analyzed in parallel (Fig. 4, bottom). Wild-type cells grown under LL intensity that contain low amounts of β-carotene did not reveal any cross-reactivity. However, exposure of these cells to HL or nitrate starvation, conditions that induce β-carotene accumulation, also induced the synthesis of Cgp. Mutant cells, which overproduce large amounts of β-carotene under noninductive conditions (LL), reacted strongly with the antibodies, suggesting that the amount of protein that these cells contain, even under noninducing conditions, is much higher than in wild-type cells. A weaker cross-reaction with the lower molecular mass polypeptide is evident in the mutant cells, which disappears when the amount of cells is reduced (Fig. 4B, lane 1/3). This cross-reaction is consistent with the idea that the lower molecular mass polypeptide is structurally related to Cgp. In *D. salina*, a species that does not accumulate β-carotene under induction conditions, there is no cross-reactivity with the antibodies, even using 10 times more cells.

Figure 5 shows the kinetics of Cgp and β-carotene synthesis under two inductive conditions. The level of Cgp increased in parallel with β-carotene accumulation under both induction conditions, HL intensity or nitrate starvation. Under nitrate starvation the major increase of both Cgp and β-carotene occurred between 24 and 48 h. Under HL, Cgp and β-carotene levels became evident already after 12 h and reached a steady state after 48 h. These results suggest that Cgp synthesis occurs in parallel with β-carotene accumulation.

Effect of Inhibition of β-Carotene Synthesis on Cgp Production

To determine whether Cgp is correlated with β-carotene accumulation or is associated with globule formation, we induced cells for β-carotene synthesis and blocked the

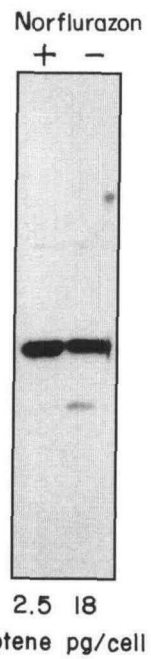

Figure 6. Accumulation of Cgp in norflurazon-treated cells. Immunoblot analysis of cells induced by nitrate starvation with and without norflurazon (3×10^{-7} M). Induction and inhibition conditions are as described in "Materials and Methods."

metabolic pathway with the inhibitor norflurazon. It was previously shown that β-carotene production is inhibited by the herbicide norflurazon (Ben-Amotz et al., 1988). Under these conditions the cells accumulate the precursor phytoene in the globules. Cells were induced by nitrate starvation in the presence or absence of norflurazon. Figure 6 demonstrates that, although norflurazon effectively blocked β-carotene synthesis, it did not inhibit Cgp production. These results indicate that Cgp is associated with the formation of the globules and not specifically with β-carotene accumulation.

Cellular Localization of Cgp

Immunoelectron microscopy was utilized to identify the cellular localization of Cgp in algae grown under different conditions (Fig. 7). Cryosections of cells fixed in glutaraldehyde and acrolein were immunolabeled with rabbit anti-Cgp antibodies, followed by gold-conjugated goat anti-rabbit IgG. In LL-grown cells (Fig. 7A) there are no globules and no labeling. The labeling in HL-grown cells (Fig. 7, B–D) was confined to the globules (Fig. 7, B and D); no labeling was observed in the Golgi or nucleus region (Fig. 7C). The globules were labeled only at their periphery (Fig. 7, D and E). Cgp localization on the globules was also shown in norflurazon-treated cells (Fig. 7E).

The Physiological Role of Cgp

We noted earlier that isolated preparations of β-carotene globules are remarkably stable. However, after treatment with detergents, the globules become unstable and sticky

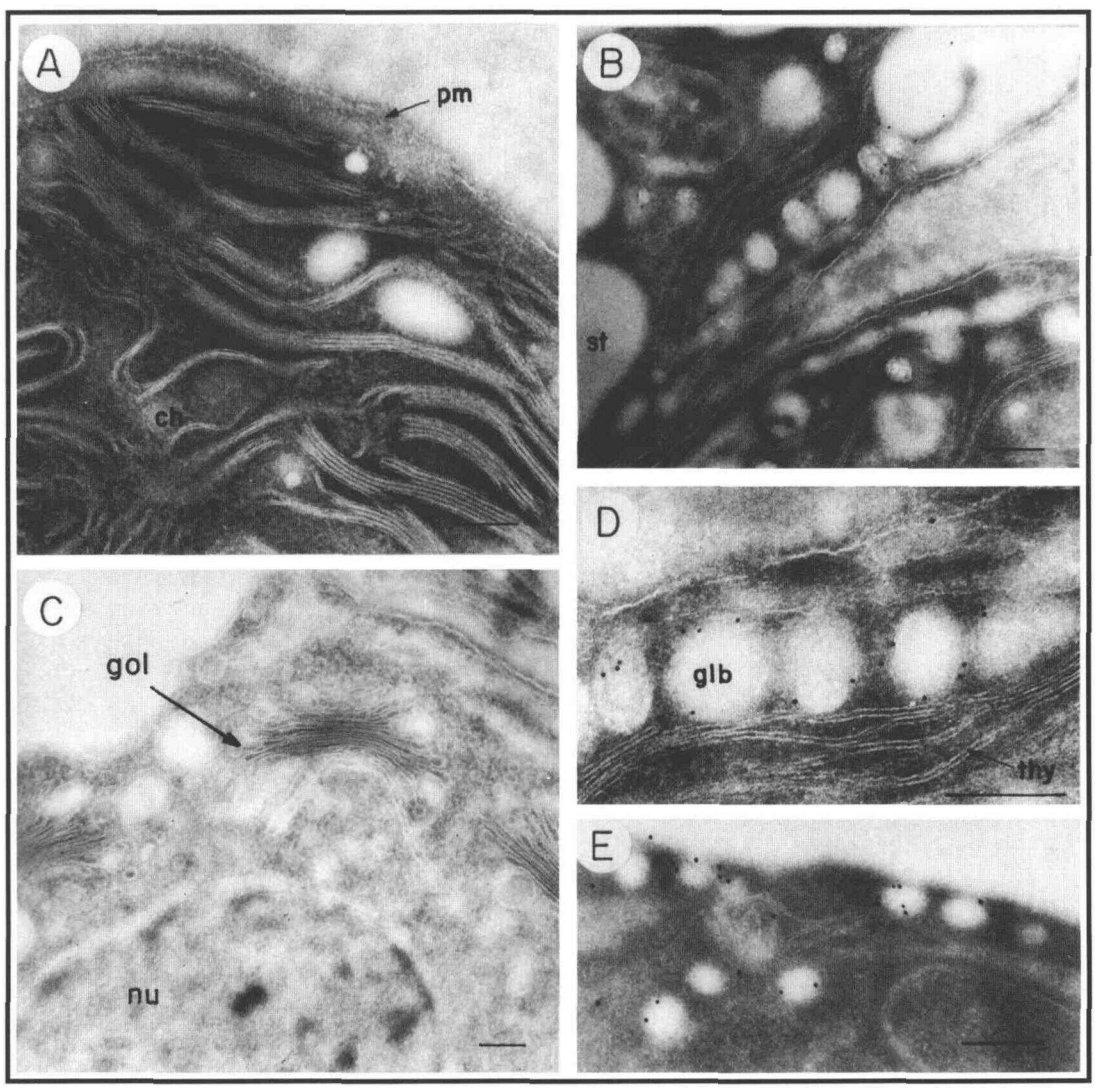

Figure 7. Electron micrographs of immunogold-labeled cells grown under various growth conditions. Cryosections were labeled with anti-Cgp antibodies diluted 1:300 at 4°C overnight, followed by a 30-min incubation at room temperature with 10-nm gold-conjugated goat anti-rabbit IgG, diluted 1:25. A, LL cell section, control for nonspecific labeling; B, HL cell section, chloroplast area; C, HL cell section, cytoplasm area; D, higher magnification of B, globule area; E, norflurazon-inhibited cell section. pm, Plasma membrane; ch, chloroplast; st, starch; gol, Golgi; nu, nucleus; glb, globules; thy, thylakoid membranes. Bar = 0.25 μm.

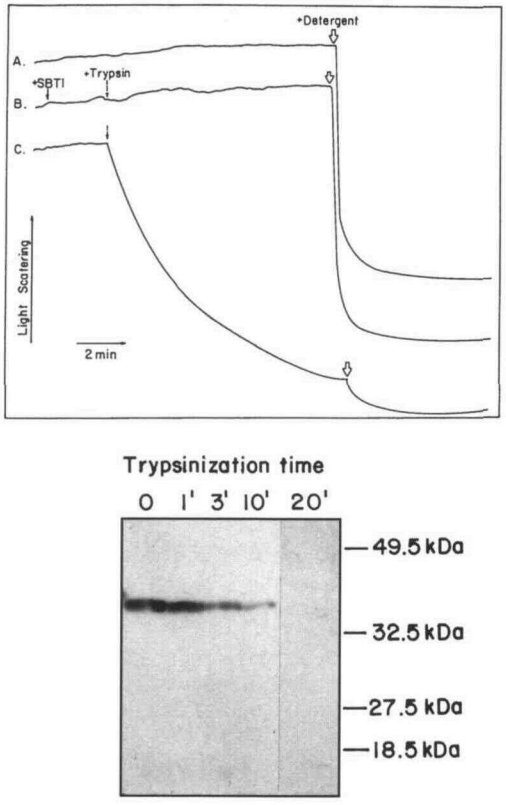

Figure 8. Effect of trypsin on globule stability and protein content. Top, Light-scattering measurements of isolated globule suspension carried out as described in "Materials and Methods." A, Intact globules; B, globules treated with SBTI before trypsin addition; and C, globules with added trypsin. Arrows indicate the addition of 40 μg/mL SBTI, 10 μg/mL trypsin, and 0.5% octyl-POE. Bottom, Immunoblot analysis of extracted globule proteins after 0, 1, 3, 10, and 20 min of trypsin treatment.

and tend to aggregate. The peripheral localization of Cgp pointed to the possibility that the protein may play a role in stabilization of the globular structure. If indeed Cgp stabilizes the structure of the globules, it may be expected that its elimination should induce their disruption. To test this possibility we assayed the effect of trypsin on globule integrity by monitoring the changes in light scattering. As demonstrated in Figure 8, trypsin treatment induced a slow decrease in light scattering (Fig. 8C), in comparison to detergent (octyl-POE) treatment, which destroyed the globules and led to a fast decrease in light scattering. Addition of SBTI before the trypsin treatment completely prevented changes in light scattering (Fig. 8B).

As shown in Figure 8, bottom, trypsinization also induced a gradual disappearance of Cgp polypeptide, consistent with the destruction of the globules.

Trypsin treatment also perturbed the spherical uniform structure of the globules and decreased their number as visualized in the electron microscope pictures of negatively stained globules (Fig. 9). These data suggest that Cgp has a role in maintaining the globule structure in *Dunaliella*.

DISCUSSION

In this work we demonstrate that β-carotene globules in *D. bardawil* possess a specific protein that accumulates in parallel with the massive accumulation of β-carotene and is associated with formation of globules. Under LL conditions the algae contain a low amount of β-carotene, which is probably part of the photosynthetic apparatus (Jiménez and Pick, 1994), globules are not observed, and Cgp is not produced. Under induction conditions, massive amounts of β-carotene accumulate within globules in the interthylakoid space, and in parallel Cgp is produced. The observation that Cgp is also produced when the metabolic pathway of β-carotene synthesis is blocked by norflurazon suggests that Cgp accumulation depends on induction conditions and globule formation rather than directly on the accumulation of β-carotene. Furthermore, Cgp is not in-

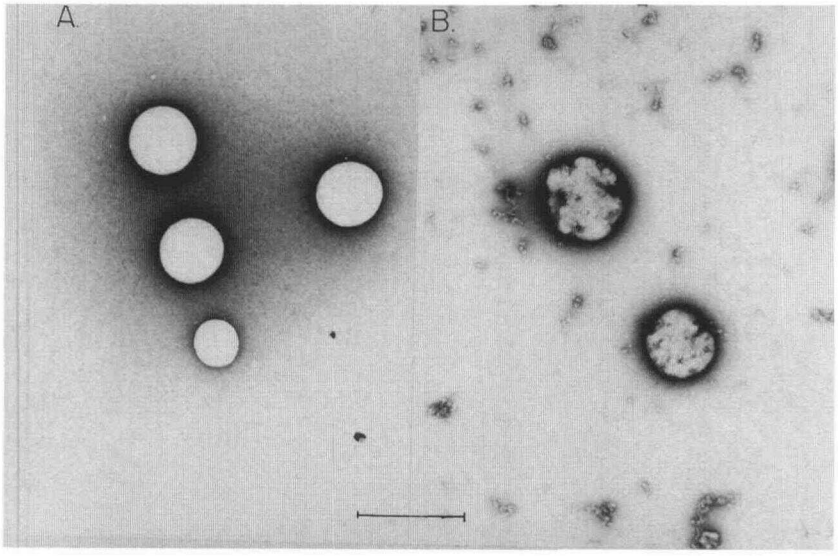

Figure 9. EM of trypsin-treated globules. Globules were treated with 10 μg/mL trypsin for 3 min and negatively stained with 2% uranyl acetate. A, Untreated globules; B, treated with trypsin. Bar = 0.5 μm.

duced in a related *Dunaliella* species that does not accumulate β-carotene.

The localization of the protein at the periphery of the globules is indicated by the EM immunolabeling and by its accessibility to trypsin. The observation that the protein can be detached from the globules by a mild detergent treatment and that salt partially strengthens the association with the globules (Fig. 2) suggests that Cgp interactions with the globules are primarily hydrophobic in nature.

The lower molecular mass protein that accompanies Cgp in the mutant globules appears to vary in amount relative to the 38-kD polypeptide depending on the induction conditions: under LL intensity it is a minor component (Fig. 4), whereas under HL induction it becomes comparable to the 38-kD polypeptide (Fig. 3). The relationship between the 38-kD and the lower molecular mass polypeptides is not clear, but we observed that fragmentation of these polypeptides with the protease *S. aureus* V8 (see "Materials and Methods") yields a similar pattern of proteolytic fragments, which have similar N-terminal amino acid sequences: 38 kD, LHDLRPCG; lower molecular mass, LHDIRPAG. This sequence similarity and antibody cross-reactivity may indicate that the lower molecular mass polypeptide is either a degradation product or an isoform of the 38-kD polypeptide.

The role of Cgp is still not clear. Its peripheral localization and the enhanced aggregation of globules following cleavage by trypsin suggest that the function of this protein may be structural stabilization of the globules. The high stability of β-carotene globules from *D. bardawil* in vivo and in vitro, in spite of their tiny dimensions and hydrophobic content, may result from Cgp, which provides a stabilizing hydrophilic layer covering the hydrophobic pigment core. A similar role was suggested for oleosins in stabilization of triacylglycerol oil bodies in seeds (reviewed by Huang, 1992) and for fibrillin in stabilization of carotenoid fibril structures in chromoplasts of bell pepper (Deruére et al., 1994). At present it is not clear whether Cgp is structurally related to oleosins or fibrillin.

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LITERATURE CITED

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