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Release of Photosynthetic Protein Catabolites by Blebbing from Thylakoids[1]

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Thylakoid proteins and their catabolites have been detected in lipid-protein particles isolated from the stroma of intact chloroplasts obtained from primary leaves of 2-week-old bean seedlings (*Phaseolus vulgaris* L. cv Kinghorn). The lipid-protein particles bear morphological resemblance to plastoglobuli seen in the chloroplasts of senescing leaves, but they are much smaller. They range from 10 to 320 nm in radius, are uniformly stained in thin sections visualized by transmission electron microscopy, and are discernible in the stroma of chloroplasts in corresponding thin-sectioned leaf tissue. The lipid-protein particles contain thylakoid lipids and are enriched in free fatty acids. Specifically, the free-to-esterified fatty acid ratio is about 1:1 in the particles compared to only 1:18 for corresponding thylakoid membranes. Western blot analyses indicate that these particles also contain thylakoid proteins and, in some cases, catabolites of these proteins including the CF_1 β and γ subunits of ATPase, cytochrome *f*, and the 31- and 33-kD proteins of PSII. Lipid-protein particles with similar properties were generated in vitro from isolated, light-stressed thylakoids. Collectively, these data suggest that blebbing of lipid-protein particles may be a means of removing potentially destabilizing macromolecular catabolites from thylakoid membrane bilayers.

Thylakoid membranes contain six major protein complexes, viz. the PSI reaction center, the PSII reaction center, the light-harvesting complexes associated with PSI and PSII, and Cyt b_6/f and the chloroplast coupling factor (CF_0 and CF_1) (Simpson and Wettstein, 1989). Fluorescence studies have shown that there are distinguishable populations of PSII centers in the granal (termed $PSII_\alpha$) and stromal (termed $PSII_\beta$) thylakoids (Anderson and Melis, 1983; Henrysson and Sundby, 1990). Indeed, the photoreduction of quinone acceptors by $PSII_\alpha$ is 2 to 3 times faster than that by $PSII_\beta$ (Melis, 1991). It is also apparent that the biosynthesis, the assembly and disassembly, and the degradation of these complexes are selectively regulated in response to environmental and developmental conditions (Galzer and Melis, 1987; Schuster et al., 1988; Sanders et al., 1989).

Reduced photosynthetic activity under adverse environmental conditions has been attributed primarily to damaged PSII systems (Berry and Bjorkman, 1980; Nash et al., 1985), whereas the PSI reaction center appears to be more resilient under such conditions (Gepstein, 1988). Under normal growth conditions, thylakoid membranes contain a small population of damaged PSII centers that are subject to turnover, but exposure to environmental stress results in enhanced catabolism of the 32-kD plastoquinone-binding reaction center polypeptide (D1), release of the extrinsic 33-, 23-, and 16-kD polypeptides into the lumenal space, and lateral migration of disassembled PSII centers from appressed to nonappressed thylakoid regions (Hundal et al., 1990; Virgin et al., 1990). The D1 polypeptide of PSII undergoes light-dependent phosphorylation (Millner et al., 1986), but it is not clear whether this is involved in D1 catabolism and whether proteolysis of D1 occurs in the grana- or stroma-exposed thylakoid regions (Aro et al., 1993). However, selective replacement of specific polypeptides within multi-protein complexes, and of complexes themselves, would appear to be essential to preclude the metabolically expensive task of dismantling and replacing entire granal structures (Melis, 1991). Indeed, it has been reported that levels of all the components of the Cyt b_6/f complex sharply decline during environmental stress, whereas levels of the PSI reaction center and of the coupling factor (CF_1) remain unchanged (Ben-David et al., 1983; Gepstein, 1988).

The lipid composition of thylakoid membranes is conserved in a wide range of plant species and is characterized by an abundance of polyunsaturated fatty acids that are prone to oxidative degradation (Girotti, 1990). Peroxidation of thylakoid lipids results in reduction of electron transport (Mishra and Singhal, 1992), and it is noteworthy in this context that oxygen is always abundant in the vicinity of functioning PSII reaction centers. Hydrolysis of MGDG and DGDG to their constitutive glycerol, Gal, and free fatty acid components is mediated by three enzymes: α-galactosidase, β-galactosidase, and galactolipase (Dalling and Nettleton, 1986). Free linolenic acid, the dominant fatty acid of thylakoid lipids, has been shown to activate Chl oxidase (Martinoia et al., 1982; Thomas, 1982), and it has been suggested that free fatty acids derived from the hydrolysis of MGDG and DGDG may also activate proteolysis within the thylakoid membrane (Dalling and Nettleton, 1986).

The fate of thylakoid lipid and protein catabolites once they are formed within the bilayer is largely unknown. Recently, lipid-protein particles that are enriched in lipid and protein catabolites have been isolated from the cytosol of plant tissue (Yao et al., 1991a, 1991b). Lipid-protein particles

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Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol.

with essentially similar properties can also be formed in vitro from isolated membranes under conditions in which phospholipid catabolism is activated (Yao et al., 1991a, 1991b). It has been proposed that these particles are formed by blebbing from membranes and may serve as a vehicle for removing destabilizing catabolites from membrane bilayers (Yao et al., 1991a, 1991b). In the present study, we isolated and partially characterized lipid-protein particles from the stroma of intact chloroplasts that have properties consistent with their prospective involvement in the removal of destabilizing catabolites from thylakoids.

MATERIALS AND METHODS

Fractionation Procedures

Bean seeds (*Phaseolus vulgaris* L. cv Kinghorn) were germinated in Pro-mix BX (Plant Products, Brampton, Ontario, Canada) at 25°C under a 16-h photoperiod (450 μmol m^{-2} s^{-1}) of mixed fluorescent and incandescent light. Chloroplasts were isolated from primary leaves of 2-week-old seedlings essentially as described by Pardo et al. (1980). Briefly, 400 g of leaf tissue were homogenized with a Sorvall Omnimixer (three 2-s periods) in buffer A (5 mL g^{-1}) comprising 100 mM Hepes-KOH (pH 8.0), 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 0.25 M sorbitol, and 1% (v/v) glycerol. The homogenate was filtered through eight layers of cheesecloth and centrifuged at 12,000*g* for 1 min. The pellet was resuspended in 200 mL of buffer A and centrifuged at 150*g* for 1 min. The supernatant was recentrifuged at 2,000*g* for 2 min, and the resulting pellets were resuspended in 40 mL of buffer A, layered in 5-mL aliquots onto 12-mL cushions of buffer A containing 6 M Suc, and centrifuged at 500*g* for 15 min in a Sorvall HB-4 rotor. The resulting pellets of intact chloroplasts were washed twice by resuspension in 20 mL of buffer A and centrifugation at 12,000*g* for 2 min.

Lipid-protein particles were isolated from the stroma of intact chloroplasts according to the protocol in Figure 1A. The chloroplasts were osmotically lysed in buffer B (200 mg Chl 200 mL^{-1} buffer) consisting of 10 mM Hepes-KOH (pH 8.0), 10 mM $MgCl_2$, and 10 mM $NaHCO_3$, and diluted with an equal volume of 2× buffer A. The suspension was then centrifuged at 12,000*g* for 10 min to yield thylakoids and stroma (Fig. 1A). Protease-inhibitor cocktail (PMSF, benzamidine, and caproic acid) was added to the stromal fluid (Fig. 1A), the mixture was centrifuged again to remove any residual membranes, and nonsedimentable lipid-protein particles were isolated from the supernatant as described previously (Yao et al., 1991a) by ultrafiltration through a 300,000- or 1,000,000-D filter (Fig. 1A).

Lipid-protein particles were also generated in vitro from isolated thylakoids exposed to light stress as shown in Figure 1B. Thylakoids were resuspended in buffer A (0.25 mg Chl mL^{-1}) and placed under light (450 μmol m^{-2} s^{-1}) for 2, 4, or 6 h. Lipid-protein particles generated under these in vitro conditions were isolated in buffer A containing protease-inhibitor cocktail by centrifugation and ultrafiltration (Fig. 1B).

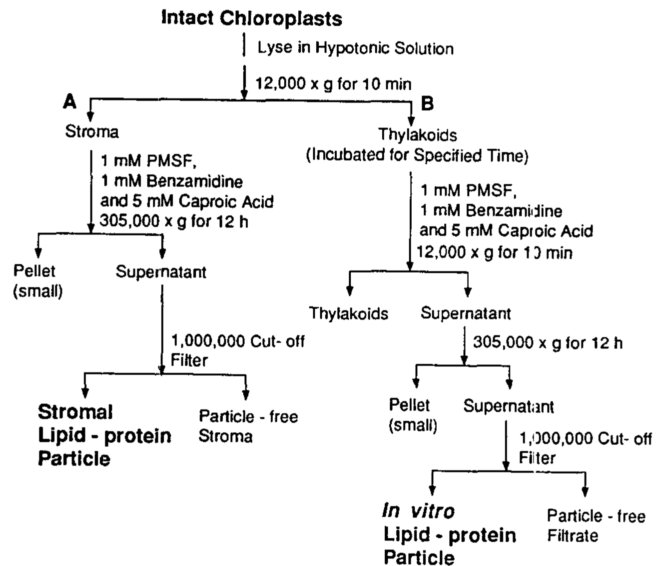

Figure 1. Isolation of lipid-protein particles from the stroma of intact chloroplasts (A) and the generation of lipid-protein particles in vitro from isolated thylakoids (B).

EM and Light-Scattering Measurements

Isolated intact chloroplasts and isolated lipid-protein particles were fixed in glutaraldehyde, immobilized in soft agar, and then fixed in osmium tetroxide, embedded in plastic, thin-sectioned, and stained for examination under a Phillips 300 transmission electron microscope operated at 60 kV (Glauert, 1965). In other experiments, isolated lipid-protein particles were mounted directly on grids, stained with uranyl acetate, air-dried, and examined under a Phillips 300 transmission electron microscope operating at 60 kV (Yao et al., 1991a). Isolated lipid-protein particles were sized by dynamic light scattering using a helium-neon laser (model 125, Spectra Physics, San Jose, CA) as described by Hallett et al. (1989).

Gel Filtration Chromatography and Biochemical and Chemical Analyses

Isolated lipid-protein particles were further purified by gel filtration chromatography using Sephacryl S-300 (Pharmacia). Two milliliters of lipid-protein particles (approximately 0.80 mg of protein) were loaded onto a 1.6-cm × 95-cm column and eluted with buffer A. Protein was quantified according to Ghosh et al. (1988) using BSA (fraction V, Sigma) as a standard. Proteins were fractionated by SDS-PAGE (Laemmli, 1970) and stained with silver (Wray et al., 1981). Selected thylakoid proteins were visualized on western blots using monospecific polyclonal antibodies (Ghosh et al., 1989). Lipids were extracted according to Bligh and Dyer (1959) and separated by TLC on Silica Gel 60 plates (Mandel Scientific, Guelph, Ontario, Canada) as described by Yao et al. (1991a). The separated lipids were identified by co-chromatography using authentic standards. Total fatty acids were measured by GLC after transmethylation using BF_3-methanol (Morrison and Smith, 1964). Rubisco activity was assayed as described by Ghosh et al. (1989).

RESULTS

Lipid-protein particles were isolated from the stroma of intact chloroplasts by ultrafiltration. This involved concentrating 200 mL of stroma, which had been rendered free of residual membrane by protracted high-speed centrifugation, to 20 mL by ultrafiltration and washing the 20 mL of suspended particles retained by the filter three times by adding 20 mL of buffer and reducing each volume to 20 mL by ultrafiltration. Thus, the procedure gave rise to a preparation of purified lipid-protein particles, which did not pass through the filter, and a filtrate consisting of particle-free stroma (Fig. 1A). In some experiments, the lipid-protein particle suspension was washed with buffer containing 3 M sodium bromide to remove any residual stromal proteins that remained ionically associated with the particle surfaces. However, this treatment did not further reduce the protein content, nor did it alter the SDS-PAGE banding pattern of the lipid-protein particle preparation (data not shown).

Isolated lipid-protein particles visualized by EM after staining with uranyl acetate were spherical in nature (Fig. 2, A and B), and they were also of variable size, suggesting either fusion or budding of smaller particles from the larger ones (Fig. 2B). Indeed, dynamic light-scattering measurements indicated that the particles range from 10 to 320 nm in radius (Fig. 3A). The isolated lipid-protein particles also appeared uniformly stained in thin section (Fig. 2C). Uniformly stained spherical particles within this size range were also evident in thin sections of corresponding intact chloroplasts (Fig. 2D). Particles were not detected in the purified stroma by light scattering or EM (data not shown). Lipid-protein particles formed in vitro from isolated thylakoids ranged from approximately 100 to 220 nm in radius (Fig. 3B).

TLC of lipid extracts from stromal and in vitro-generated lipid-protein particles indicated that they contain MGDG and DGDG, the major thylakoid lipids, as well as diacylglycerol and free fatty acids (Fig. 4). The lipid composition of the lipid-protein particles, however, is clearly distinguishable from that of thylakoids. In particular, there is approximately an 18-fold enrichment of free relative to esterified fatty acids in the particles compared to thylakoids. Specifically, the free-

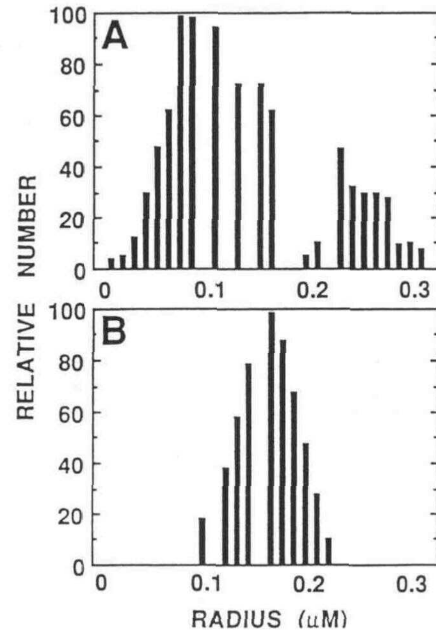

Figure 3. Light-scattering measurements of the size distribution of stromal lipid-protein particles (A) and in vitro-generated lipid-protein particles (B). Data are from one of three separate experiments showing the same results.

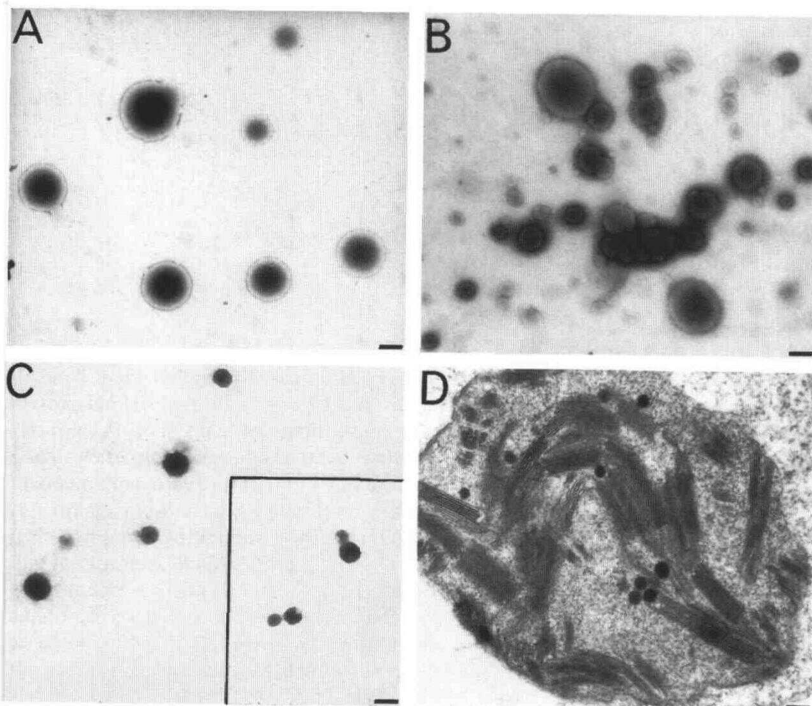

Figure 2. Transmission EM of uranyl acetate-stained mounts of stromal lipid-protein particles (bar indicates 70 nm) (A and B); thin-sectioned stromal lipid-protein particles (bar indicates 140 nm; inset illustrates associated particles) (C); and thin-sectioned chloroplasts (bar indicates 140 nm) (D).

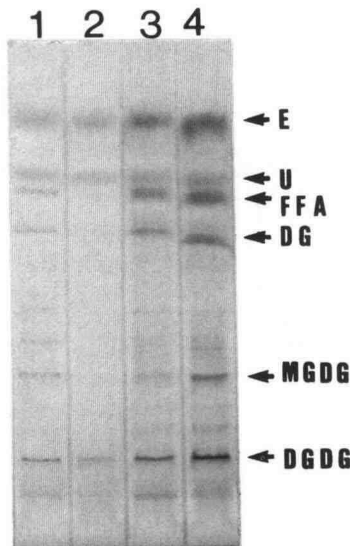

Figure 4. TLC of total lipid extracts from stromal and in vitro-generated lipid-protein particles. Lane 1, Lipids from stromal lipid-protein particles isolated from intact chloroplasts (50 mg of Chl); lanes 2 through 4, lipids from in vitro-generated lipid-protein particles formed from thylakoids (50 mg of Chl equivalents) during illumination for 2, 4, and 6 h, respectively. E, Esters; U, unknown; FFA, free fatty acids; DG, diacylglycerols. Data are from one of three separate experiments showing the same results.

to-esterified fatty acid ratio was 1.06 ± 0.04 (SE) in the lipid-protein particles and only 0.06 ± 0.003 (SE) in thylakoids. There was also an increase in the formation of in vitro-generated lipid-protein particles with time of incubation as reflected by increased levels of lipid (Fig. 4, lanes 2–4), indicating that the formation of lipid-protein particles from thylakoids under in vitro conditions is time dependent. These data were obtained by illuminating the thylakoid suspension. In the absence of light, the yield of lipid-protein particles was reduced by approximately 50%, and prior heat denaturation (10 min at 100°C) of the thylakoids inhibited the formation of lipid-protein particles by 98%. Additionally, illumination (175 μmol m^{-2} s^{-1}) of isolated chloroplasts in buffer A (8 μg Chl mL^{-1}) for 1 h increased the yield of isolated lipid-protein particles by approximately 65%.

The finding that lipid-protein particles generated from thylakoids under in vitro conditions have the same lipid composition as those isolated from the stroma of intact chloroplasts suggests that the stromal lipid-protein particles are formed by blebbing from thylakoids. This contention is further supported by western blot analyses showing that stromal lipid-protein particles and those formed in vitro from thylakoids both contain thylakoid photosynthetic proteins and, in some cases, catabolites of these proteins. Specifically, the 56-kD β subunit of ATPase was detected in untreated thylakoids as well as in thylakoids that had been illuminated in buffer for 2 h to generate in vitro lipid-protein particles (Fig. 5A, lanes 1 and 2). However, a 54-kD catabolite of the β subunit of ATPase rather than the native protein was detectable in both stromal and in vitro lipid-protein particles (Fig. 5A, lanes 3 and 5), and neither the native protein nor its catabolites were detectable in particle-free stroma or in the in vitro filtrate (Fig. 5A, lanes 4 and 6). By contrast, the 37-kD γ subunit of ATPase was present in the thylakoids (Fig. 5B, lanes 1 and 2) and in stromal and in vitro lipid-protein particles (Fig. 5B, lanes 3 and 5), and again it was not detectable in particle-free stroma or in the in vitro filtrate (Fig. 5B, lanes 4 and 6). Cyt *f* (34 kD) was present in thylakoids (Fig. 5C, lanes 1 and 2), but stromal lipid-protein

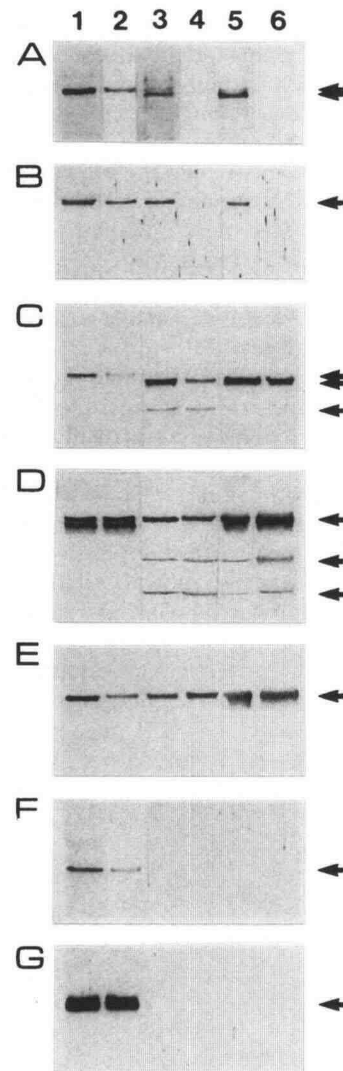

Figure 5. Immunological identification of thylakoid proteins and their catabolites in western blots prepared from thylakoids (lane 1), thylakoids after illumination for 2 h to generate in vitro lipid-protein particles (lane 2), stromal lipid-protein particles (lane 3), particle-free stroma (lane 4), in vitro-generated lipid-protein particles (lane 5), and particle-free supernatant from the in vitro generation of particles (lane 6). The blots were treated with antisera against CF_1 β subunit of ATPase (56 kD) (A); CF_1 γ subunit of ATPase (37 kD) (B); Cyt *f* (34 kD) (C); 33-kD protein of PSII (D); D2 protein of PSII (31 kD) (E); light-harvesting complex of PSII (26 kD) (F); and PSI core protein (26 kD) (G). The locations of these thylakoid proteins and their catabolites are indicated by arrows. Data are from one of three separate experiments showing the same results.

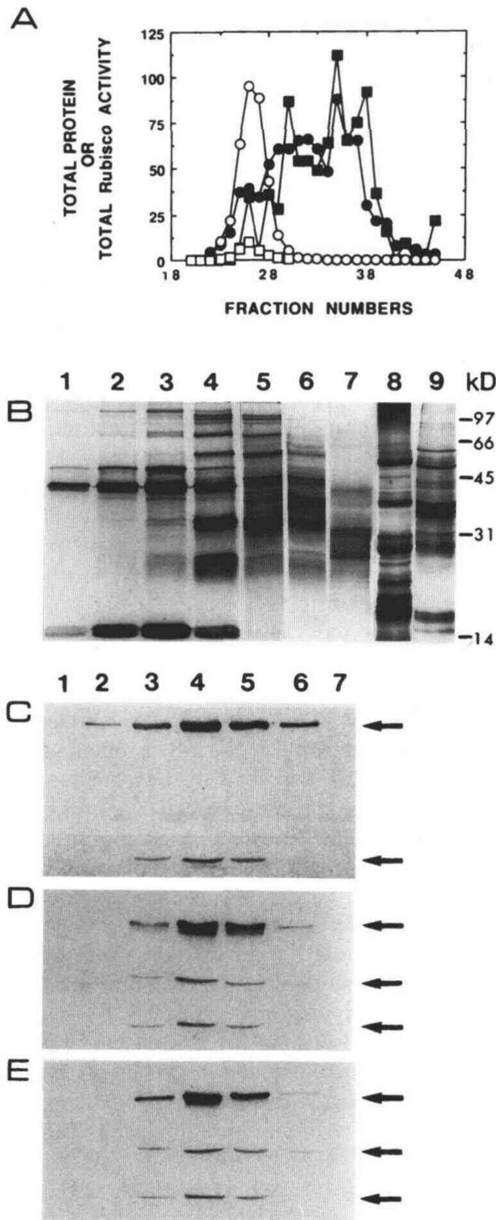

Figure 6. Sephacryl chromatography of lipid-protein particles. A, Profiles of eluted protein (μg fraction^{-1}; ●, stromal; ■, in vitro-generated) and Rubisco (dpm $\times$ 10^{-3} fraction^{-1}; ○, stromal; □, in vitro-generated). B, SDS-PAGE of eluted lipid-protein particles. Lane 1, Combined fractions 22 and 23; lane 2, combined fractions 24 and 25; lane 3, combined fractions 26 and 27; lane 4, combined fractions 28 and 29; lane 5, combined fractions 30 and 31; lane 6, combined fractions 32 and 33; lane 7, combined fractions 34 and 35; lane 8, thylakoid membranes; and lane 9, unfractionated in vitro-generated lipid-protein particles. Lanes 1 through 7 were loaded with equal volume of column fractions, lanes 8 and 9 were loaded with 1 μg of protein and the gels were stained with silver. C, Western blot detection of the large (top arrow) and small (bottom arrow) subunits of Rubisco in eluted stromal lipid-protein particles. Lane 1, Combined fractions 20 and 21; lane 2, combined fractions 22 and 23; lane 3, combined fractions 24 and 25; lane 4, combined fractions 26 and 27; lane 5, combined fractions 28 and 29; lane 6, combined fractions 30 and 31; and lane 7, combined fractions 32 and 33. D, Western blot detection of the 33-kD protein of PSII (top arrow) and its catabolites (lower arrows) in eluted stromal lipid-protein particles. Lane 1, Combined fractions 28 and 29; lane 2, combined fractions 30 and 31; lane 3, combined fractions 32 and 33; lane 4, combined fractions 34 and 35; lane 5, combined fractions 36 and 37; lane 6, combined fractions 38 and 39; and lane 7, combined fractions 40 and 41. E, Western blot detection of the 33-kD protein of PSII (top arrow) and its catabolites (lower arrows) in eluted in vitro-generated lipid-protein particles. Lanes are as in D.

particles and particle-free stroma contained only 31- and 26-kD catabolites of this protein (Fig. 5C, lanes 3 and 4). The in vitro lipid-protein particles generated from isolated thylakoids exposed to light stress and the corresponding filtrate had detectable levels of the 31-kD catabolite of Cyt *f*, but the 26-kD catabolite was not present (Fig. 5C, lanes 5 and 6). As noted previously (Roberts et al., 1987), the 33-kD protein of PSII appeared as a doublet in the thylakoid membranes (Fig. 5D, lanes 1 and 2), but only the upper band of the doublet was clearly discernible in the lipid-protein particles, particle-free stroma, and in vitro filtrate (Fig. 5D, lanes 3–6). Two catabolites of the 33-kD protein of PSII were also detected in the lipid-protein particles (Fig. 5D, lanes 3 and 5) and in the particle-free stroma and in vitro filtrate (Fig. 5D, lanes 4 and 6). The 31-kD (D2) protein of PSII was present in all of the fractions, but catabolites of this polypeptide were not detected (Fig. 5E). The 26-kD protein (LHCP) of PSII and the 26-kD core protein of PSI were present in the thylakoids, but neither the native proteins nor their catabolites were detected in the lipid-protein particles, the particle-free stroma, or in the in vitro filtrate (Fig. 5, F and G).

SDS-PAGE indicated that the polypeptide profile of the stromal lipid-protein particles is complex (Fig. 6B, lane 5) and clearly distinguishable from that of corresponding thylakoid membranes (Fig. 6B, lane 8). The polypeptide composition of in vitro-generated lipid-protein particles (Fig. 6B, lane 9) was similar, although not identical, to that of stromal lipid-protein particles (Fig. 6B, lane 5) and again clearly distinguishable from that of corresponding thylakoid membranes (Fig. 6B, lane 8). Polypeptide bands corresponding to the large and small subunits of Rubisco were also evident in the stromal lipid-protein particle fraction, indicating contamination by stromal protein (Fig. 6B). This was particularly evident when a 300,000-D filter was used for isolating the lipid-protein particles from the stroma. However, Rubisco could be separated from the lipid-protein particles through further purification by Sephacryl chromatography. This was confirmed by measurements of Rubisco activity in the protein-containing fractions eluted from the column (Fig. 6A), by SDS-PAGE of the separated fractions (Fig. 6B), and by western blots using monospecific polyclonal antibodies raised against the large and small subunits of Rubisco (Fig. 6C). It was also apparent from western blot analyses that thylakoid proteins and their catabolites were detectable in the eluted fractions containing purified lipid-protein particles and not in the contaminating Rubisco protein peak. Additionally, native thylakoid proteins and their catabolites co-eluted during the column purification of the lipid-protein complexes, confirming that they are in a complex. This is illustrated for the 33-kD protein of PSII in

Figure 6, D and E. The free-to-esterified fatty acid ratio of the column-purified lipid-protein particles was again 1.01 compared to only 0.06 for thylakoid membranes, and the column-purified lipid-protein particles were spherical and comparable in size to those loaded on the column (data not shown).

DISCUSSION

Lipid-protein particles isolated from the stroma of intact chloroplasts contain thylakoid photosynthetic proteins and, in some cases, catabolites of these proteins, but have an overall polypeptide composition that is distinguishable from that of thylakoid membranes. The lipid-protein particles are also distinguishable from thylakoid membranes on the basis of their lipid composition and, in particular, their high concentration of free fatty acids. Indeed, the ratio of free to esterified fatty acids in the lipid-protein particles proved to be 1.06 compared to a corresponding ratio of only 0.06 for thylakoids. This high concentration of free fatty acids presumably also accounts, at least in part, for the nonsedimentable nature of these lipid-protein particles.

Several lines of evidence support the view that these stromal lipid-protein particles are formed in situ by blebbing from thylakoids. First, they are isolated from the stroma of intact chloroplasts and contain MGDG and DGDG, the two dominant lipid species of the thylakoid membrane. Second, essentially similar lipid-protein particles can be generated in vitro from isolated, light-stressed thylakoid membranes, and their formation under these conditions is time dependent and inhibited by prior heat denaturation of the thylakoids. The in vitro-generated lipid-protein particles have the same lipid composition as those isolated from the stroma of chloroplasts, and western blot analyses indicate that they also contain photosynthetic proteins and their catabolites in the same pattern that was evident for stromal lipid-protein particles. Of particular interest is the finding that not all of the thylakoid photosynthetic proteins tested by western blotting were present in the lipid-protein particles, which suggests that the blebbing process is selective in nature. This view is reinforced by the fact that the lipid-protein particles do not contain Chl, although the prospect that catabolites of Chl may be present has not been examined. It is not clear what prompts the blebbing process, but the observation that stromal and in vitro-generated lipid-protein particles both contain diacylglycerol may be significant because diacylglycerol has been shown to promote microvesiculation from erythrocytes (Allan et al., 1975, 1976). Finally, the fact that these lipid-protein particles are isolated from stroma obtained by lysis of intact chloroplasts suggests that they are not homogenization artifacts. Indeed, this contention is supported by the observation that uniformly stained particles of a similar size and morphology are evident in the stroma of thin-sectioned intact chloroplasts, often in close proximity to thylakoids.

Plastoglobuli are also thought to originate from thylakoids and have morphological features in common with the lipid-protein particles of chloroplasts from young leaves characterized in the present study in that they are also osmiophilic spherical bodies present in the chloroplast stroma (Greenwood et al., 1963; Hansmann and Sitte, 1982; McRae et al., 1985; Steinmuller and Tevini, 1985; Ronning et al., 1991). However, plastoglobuli are present in the chloroplasts of senescing leaves, and their formation is thought to be associated with the breakdown of thylakoids that accompanies senescence (McRae et al., 1985; Steinmuller and Tevini, 1985; Ronning et al., 1991). Their composition is not completely known, but it has been reported that they contain prenyl quinones, carotenoids, glycerolipids, and protein (Greenwood et al., 1963; Hansmann and Sitte, 1982; Steinmuller and Tevini, 1985). Plastoglobuli are also much larger than the lipid-protein particles isolated in the present study, ranging from 80 to 1250 nm in diameter (Steinmuller and Tevini, 1985). Despite these differences, however, it remains possible that the lipid-protein particles isolated in the present study from young nonsenescing leaves are fundamentally similar to plastoglobuli. It would appear that plastoglobuli are formed during actual dismantling of thylakoids (McRae et al., 1985; Steinmuller and Tevini, 1985; Ronning et al., 1991), whereas formation of the lipid-protein particles in young chloroplasts may be an inherent feature of normal thylakoid membrane turnover.

It has been suggested previously that protein and lipid impurities may adsorb to plastoglobuli during their isolation (Steinmuller and Tevini, 1985). In the present study, preparations of stromal lipid-protein particles did contain Rubisco, which could be removed by Sephacryl chromatography, indicating that it was not adsorbed to the particles. As well, particles of similar size and morphology containing the same lipids, as gauged by TLC, the same high proportion of free to esterified fatty acids, and the same pattern of photosynthetic electron transport proteins and their catabolites can be formed under in vitro conditions from purified thylakoid membranes. This supports the contention that these lipids and proteins are inherent components of the particles.

Nonsedimentable lipid-protein particles have also been isolated from the cytosol of plant tissue (Yao et al., 1991a, 1991b) and rat liver (Yao et al., 1993). These cytosolic lipid-protein particles resemble chloroplast stromal lipid-protein particles in that they contain protein catabolites and are enriched in free fatty acids, but they also contain phospholipids rather than galactolipids (Yao et al., 1991a, 1991b, 1993). Nonsedimentable lipid-protein particles resembling those isolated from the cytosol can also be formed in vitro from isolated microsomal membranes under conditions in which phospholipid catabolism has been activated by the addition of Ca^{2+} (Yao et al., 1991a, 1993). It has been proposed that these cytosolic lipid-protein particles are formed by blebbing from membranes and may serve as a vehicle for removing catabolites from membranes that would otherwise destabilize the bilayer (Yao et al., 1991a). They have been termed deteriosomes to connote this putative function (Yao et al., 1991a, 1991b, 1993). It is conceivable that the lipid-protein particles present in the stroma of nonsenescing chloroplasts play a similar role in removing destabilizing lipid and protein catabolites from thylakoids. This view is supported by the fact that they are enriched in free fatty acids, a major galactolipid catabolite, and that they contain catabolites of certain thylakoid photosynthetic proteins, notably the β subunit of ATPase, Cyt *f*, and the 33-kD protein of PSII that are not detectable in thylakoid membranes.

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