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DISSOCIATION OF SUPRAMOLECULAR COMPLEXES IN CHLOROPLAST MEMBRANES

A MANIFESTATION OF HEAT DAMAGE TO THE PHOTOSYNTHETIC APPARATUS *

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Summary

High temperature-induced alterations to membrane structure were investigated for chloroplast thylakoid membranes isolated from leaves of *Nerium oleander* grown at a 20/15°C or 45/32°C day/night temperature regime and pretreated at temperatures from 40 to 55°C. Quantitative analysis of micrographs of freeze-fractured membranes revealed a progressive loss of exoplasmic fracture face (EF) particles from the larger particle size classes as the temperature of the pretreatment was increased. This loss indicates that the components of the EF particles, presumed to be the chlorophyll *a/b* light-harvesting complex and the photosystem II core complex become physically dissociated as a result of the heat pretreatment. The high-temperature stability of this supramolecular complex is enhanced in the samples from the plants grown at the higher temperature regime. These results demonstrate that the heat-induced damage to the photosynthetic apparatus involves not only a functional dissociation of the chlorophyll *a/b* light-harvesting complex from the photosystem II complex, but a physical dissociation as well.

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Abbreviation: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

Introduction

Plants subjected to high temperatures suffer a loss of photosynthetic activity attributable to a dysfunction of both biochemical and biophysical processes [1—6]. A significant drop in the activity of both membrane-bound and of soluble enzyme systems in heat-stressed plants has been observed. In particular, photophosphorylation and photosynthetic electron-transport rates are reduced, and the activities of certain soluble enzymes involved in CO_2 fixation decline (particularly those which require reducing power from the light reactions for activation [2,6]).

These losses of enzymatic activity in heat-damaged chloroplasts are accompanied by a loss of efficiency in the transfer of excitation energy between pigment-protein complexes in the membranes, as well as between pigment molecules [2,3]. Such alterations in energy-transfer efficiency have the direct consequence of affecting the quantum yield of electron transport, and hence of the overall process of photosynthesis [4,6]. These changes in excitation-energy transfer, detected as alterations in the chlorophyll fluorescence-emission characteristics of the chloroplast [2,3] or intact leaves [2,7], have been interpreted to indicate a functional dissociation of the bulk light-harvesting chlorophylls from the reaction center complexes [2,3].

The temperature at which permanent damage to the photosynthetic apparatus is detected appears to be characteristic of a given plant. Plants growing in continually cool environments, or those growing only during cool seasons, have a lower threshold temperature than plants growing in warmer environments or seasons [4,5]. There are certain plants that possess the ability to acclimate photosynthetically to a wide range of growth temperatures, shifting this threshold temperature in response to changes in the prevailing growth temperature [1,8]. Investigations on plants capable of such acclimation have shown that changes in the high-temperature stability of photosynthesis can be attributed primarily to an alteration in the stability of the chloroplast membranes [2,6].

In the present study, we have utilized *Nerium oleander*, a plant possessing a large potential for photosynthetic temperature acclimation, for investigations of the possible membrane structural changes that may accompany the physiological damage of the chloroplast membranes by high temperatures. With this plant material, it is possible to compare not only heat-damaged to non-heat-damaged material, but also to compare the structures of membranes possessing different physiological heat stabilities.

Previous studies of freeze-fractured chloroplast membranes have indicated that the particles of the exoplasmic fracture face (EF) correspond to the photochemically active core complexes of photosystem II surrounded by aggregates of the chlorophyll *a/b* light-harvesting pigment-protein complex (Chl *a/b* light-harvesting complex [9]). Since the interpretation of the fluorescence changes indicates a functional dissociation of these integral membrane components [3], we have concentrated our attention on the particles of the EF face.

Materials and Methods

Ramets of *N. oleander* were grown in controlled-environment chambers under a day/night temperature regime of 20/15°C or 45/32°C and a 14 h photoperiod. Excised leaves of similar age and development were pretreated at temperatures from 40 to 55°C under conditions of high humidity (98% relative humidity) and a light intensity of 135 nE · cm^{-2} · s^{-1}. The leaf temperature was first kept at 35°C for 20 min under the above conditions of humidity and light, then rapidly (less than 1 min) raised to the treatment temperature. The leaf temperature was recorded every 2 min; the average treatment temperature is noted in the figures. After maintaining the leaf at the elevated temperature for 10 min, it was rapidly cooled to 5°C, and chloroplast membranes were isolated by grinding the leaves in a medium containing 400 mM sorbitol, 100 mM sodium-Tricine (pH 7.8), 50 mM sodium ascorbate, 5 mM $MgCl_2$ and 2.5 mg/ml bovine serum albumin. The brei was filtered through two layers of Miracloth and centrifuged at 1000 × *g* for 10 min. The supernatant was discarded, and the pellet was divided into two portions. For samples in which the grana structure was to be maintained, the pellet was resuspended in 400 mM sorbitol, 5 mM sodium-Tricine (pH 7.8), 5 mM sodium ascorbate, 5 mM $MgCl_2$, 10 mM KCl, and 5 mg/ml bovine serum albumin. Where samples were to be experimentally unstacked, the pellet was resuspended in 150 mM NaCl and centrifuged at 1000 × *g* for 10 min. The pellet was resuspended in 50 mM sodium-Tricine (pH 7.8) and incubated at 5°C for 1 h. Glycerol (final concentration of 35%) was added to the resuspended membranes (both stacked and unstacked) with stirring over a 30 min period. The chloroplast membranes were centrifuged at 10 000 × *g* for 15 min, and the pelleted membranes were prepared for freeze-fracture as described [10]. Particle-size and density measurements were made on eight to twelve micrographs enlarged to × 200 000 as described [9]. Because of heat-induced distortions of the grana/stroma lamellae architecture, quantitative analysis of the micrographs (particle-size and density measurements) were performed on the more homogeneous material provided by the unstacking process. Nomenclature of fracture faces is according to that of Branton et al. [11]. For the purpose of comparison, the original particle-size histograms were normalized to 735 particles.

Results

A qualitative examination of the micrographs revealed an essentially normal freeze-fracture morphology for the 45/41.6 sample (Fig. 1) as well as the 45/49.2 and 20/40.7 samples (the samples are designated by the daytime growth temperature, 20 or 45°C, followed by the average temperature of the pretreatment). Measurements of the photosynthetic activity of these membrane samples have demonstrated either a lack (45/41.6, 20/40.7) or only slight (45/49.2) irreversible inhibition (see Ref. 6). The fracture face of the outer membrane leaflet (protoplasmic face or PF) and the fracture face of the inner membrane leaflet (exoplasmic face or EF) exhibited a clear differentiation into stacked (EF_s, PF_s) and unstacked (EF_u, PF_u) membrane regions. The particles of the EF face, considered to be composed of the photosystem

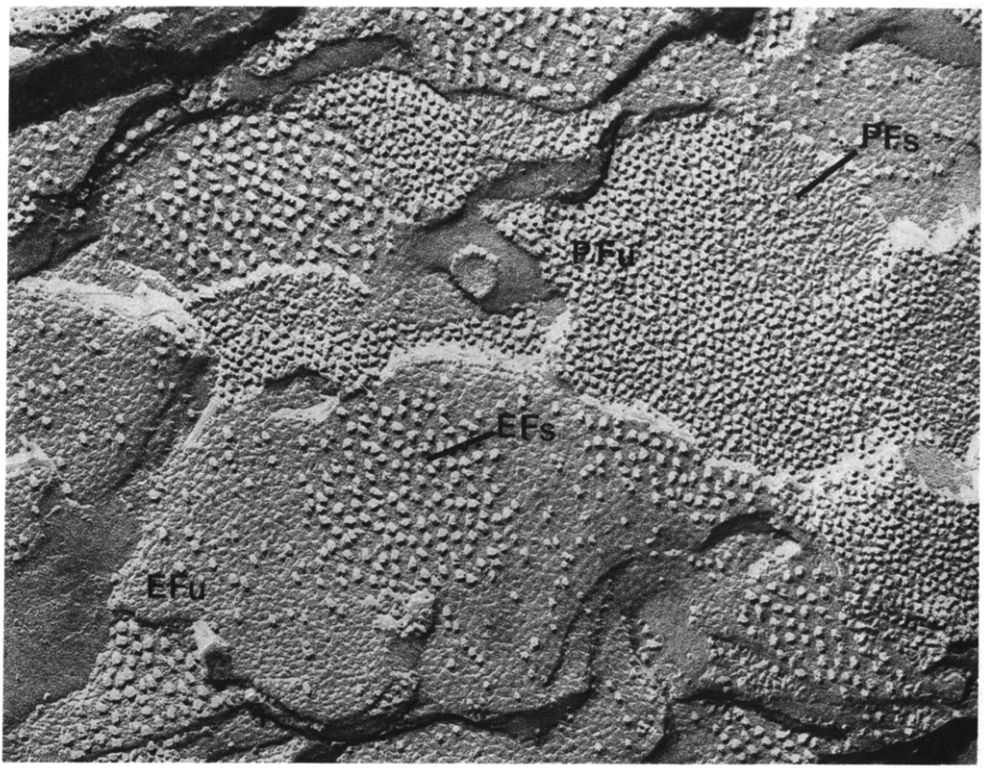

Fig. 1. Freeze-fractured thylakoid membranes isolated from leaves of *N. oleander*. A normal freeze-fracture morphology was exhibited by the 45/41.6 sample, with a clear differentiation of the membrane into stacked (EF_s, PF_s) and unstacked (EF_u, PF_u) regions. Magnification, × 100 000.

II core surrounded by the Chl *a/b* light-harvesting complex, were densely clustered into the partition regions (grana) of these chloroplast membranes, showing a normal morphological configuration. The chloroplasts of the remaining samples (20/48.8, 20/55.2, 45/55.5) demonstrate different extents of heat-induced membrane distortion. The most severe changes occurred in the 20/55.2 sample (Figs. 2 and 3). An examination of Fig. 2 reveals that the normal 'coinstack' grana have been replaced by long regions of membrane appression. Other heat-induced anomalies in the chloroplast structure were the appearance of myelin figures contiguous with the plastid membranes (Fig. 3), presumably formed by the coalescing of lipids which had leaked out of the membrane. The other membrane samples (45/55.2 and 20/49.2) exhibited the same types of morphological changes, but they were less severe and less frequent.

Quantitative analysis of freeze-fracture micrographs (particle-size frequency and particle density) requires that an unambiguous identification of the fracture faces be made. Because of the altered morphology of the heat-damaged samples, particularly with regard to the grana/stroma lamellae configuration, the quantitative analyses were performed on experimentally unstacked membranes for all samples. After such manipulation (Fig. 4), the EF_s particles, which are normally aggregated into the stacked regions of the chloroplast

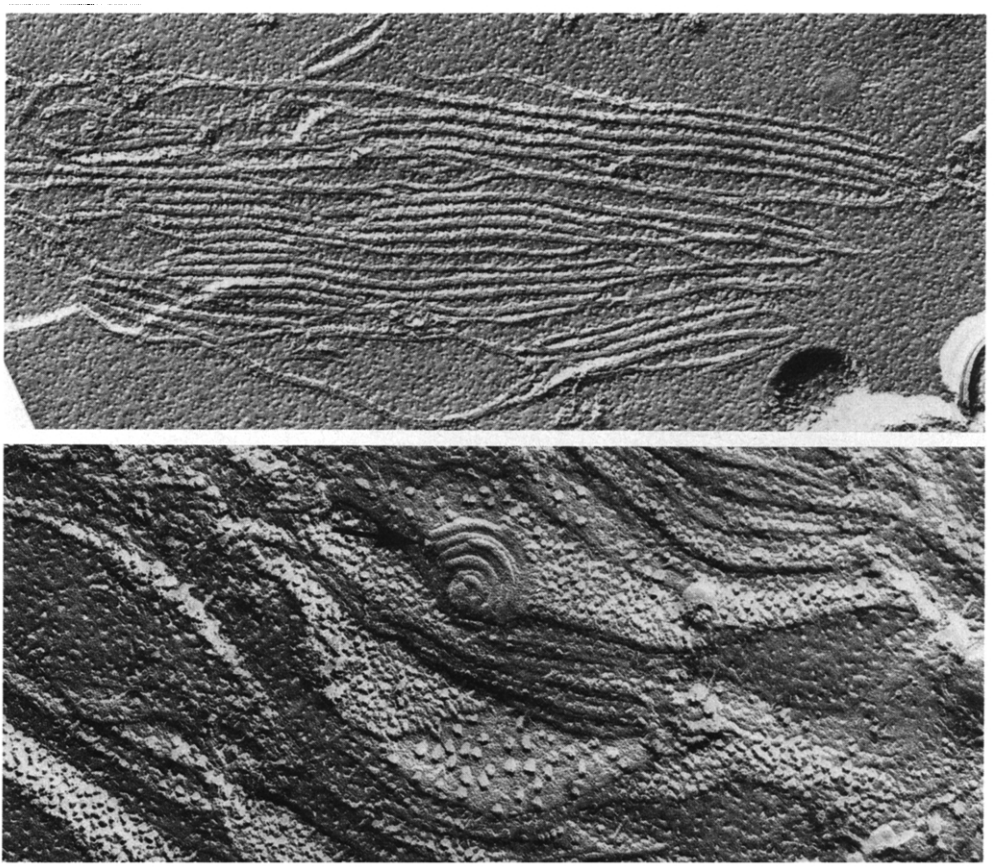

Fig. 2. Freeze-fracture of heat-damaged membranes from the 20/55.2 sample exhibiting distortion of grana stacks into long appressed regions (observed in cross-fracture). Magnification, × 60 000.

Fig. 3. Heat-damaged thylakoid membranes showing the appearance of pure lipid myelin figures contiguous with the thylakoid membranes, indicative of severe membrane damage. Magnification, × 100 000.

membrane, become intermixed with the EF_u particles and homogeneously distributed on the EF face.

Particle-size frequency histograms for the samples from the 20°C-grown material demonstrate a progressive loss of EF particles from the larger (greater than 90 Å) particle-size classes as the temperature of the pretreatment was increased (Fig. 5). This loss indicates that the components of the EF particles, presumed to be the light-harvesting complex and the photosystem II core complex, may become physically dissociated as a result of the heat pretreatment. The extent of this dissociation is more evident in the difference histograms of Fig. 6 obtained by subtracting the 20/40.7 histogram from the 20/48.8 and 20/55.2 histograms. A substantial decline in the number of particles in size classes greater than 90 Å diameter occurs with pretreatment at 48.8°C (Fig. 6, 20/48.8—20/40.7); the magnitude of this loss increases further with pretreatment at 55.2°C (Fig. 6, 20/55.2 — 20/40.7). The corresponding analyses of the particle-size frequency for the 45°C-grown samples (Fig. 7) show that

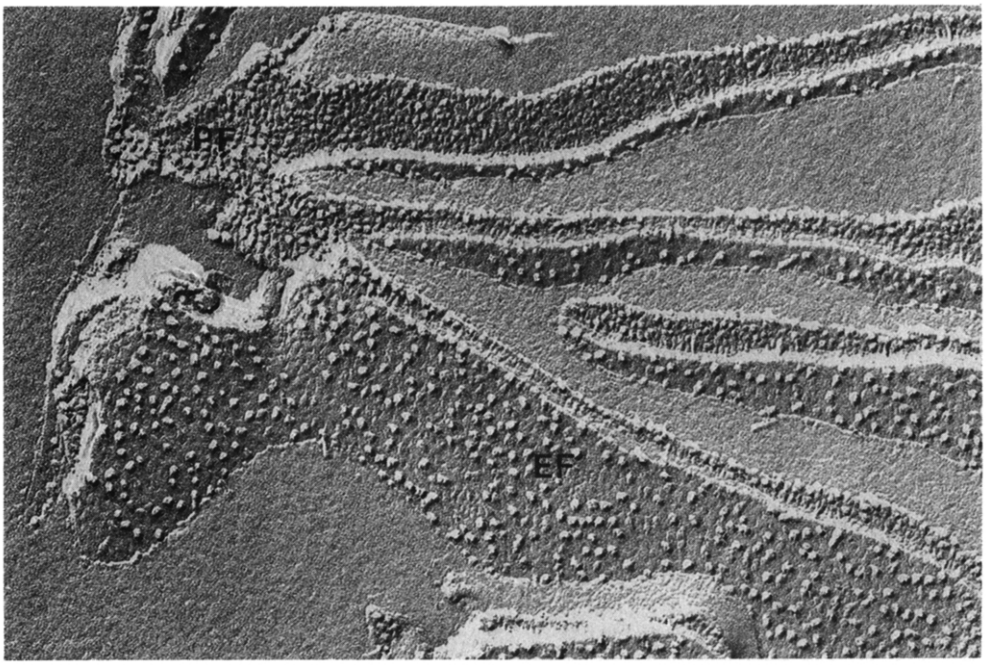

Fig. 4. Experimentally unstacked thylakoid membranes (45/41.6 sample) exhibiting an even distribution of particles on both EF and PF faces (compare with Fig. 1). Magnification, X 105 000.

the morphology of these chloroplast membranes was much less affected by the heat treatment of the leaves. The histogram of the EF particle-diameter frequency of the 45/49.2 sample displayed no substantial differences from the 45/41.6 control; the 45/55.5 sample, however, did exhibit some loss of particles in the larger diameter size classes. The difference histograms for these samples (Fig. 8) emphasize this enhanced stability of the EF particles. No obvious alteration trend is displayed in the 45/49.2—45/41.6 histogram; the 45/55.5—45/41.6 histogram shows a loss of particles in the larger size catego-

TABLE I

PARTICLE DENSITIES OF THE EF AND PF FRACTURE FACES OF *N. OLEANDER* THYLAKOID MEMBRANES

The chloroplasts were isolated from leaves pretreated at the indicated temperatures and experimentally unstacked

Growth (°C) temperature	Treatment (°C) temperature	Particles per μm^2 ± S.D.	
		EF	PF
	40.7	1656 ± 162	5433 ± 280
20	48.8	1665 ± 178	5677 ± 291
	55.2	1629 ± 176	5843 ± 315
	41.6	1764 ± 143	5789 ± 385
45	49.2	1783 ± 154	5787 ± 381
	55.5	1715 ± 143	5876 ± 385

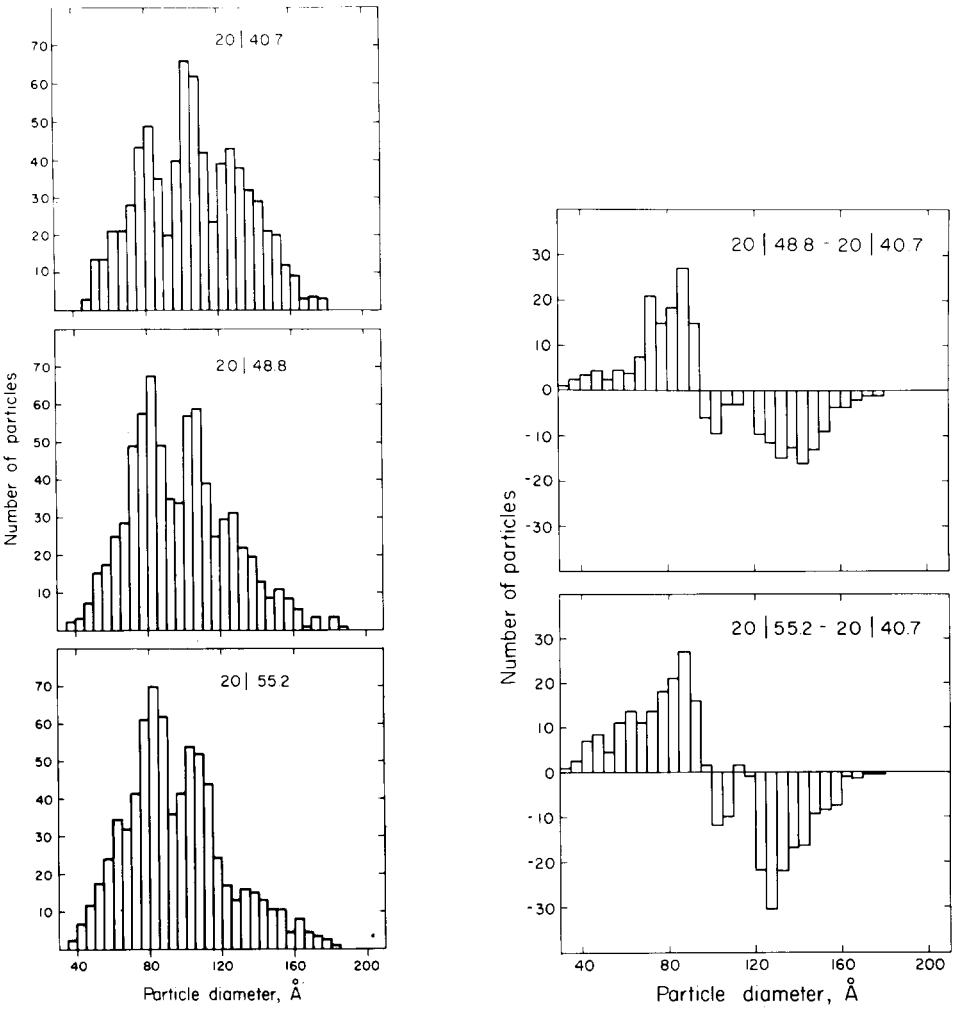

Fig. 5. Histograms of particle-size classes on the EF fracture face of chloroplasts isolated from 20°C-grown *N. oleander* leaves pretreated at 40.7, 48.8, or 55.2°C. Note the progressive loss of particles from the larger size classes with increasing pretreatment temperature. Number of particles measured: 20/40.7, 734; 20/48.8, 673; 20/55.2, 640.

Fig. 6. Difference histograms produced by subtracting the 20/40.7 histogram from the 20/48.8 and 20/55.2 histograms of Fig. 5.

ries of an extent similar to that observed in the 20/48.8—20/40.7 histogram (Fig. 6).

The particle-density measurements (Table I) do not reflect, in a significant manner, the large changes observed in the particle-size measurements. In all samples, the density of EF particles remained constant, while the number of PF particles appeared to increase slightly. It should be stressed, however, that particle-density and size measurements on PF faces are difficult to make because of the very close packing of these particles and the heat-induced distortions of the membranes. Thus, it was not possible to determine whether

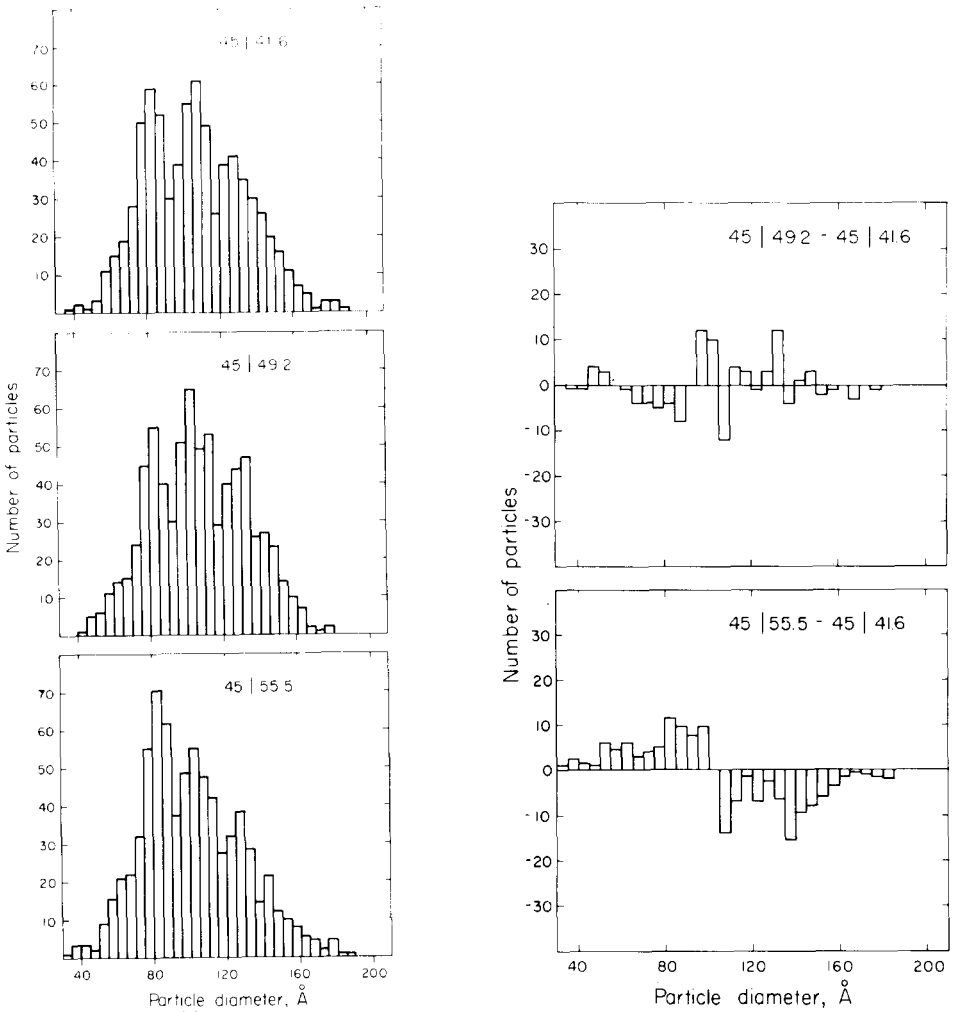

Fig. 7. Particle-size histograms for the EF fracture face of chloroplasts isolated from leaves of *N. oleander* grown at 45° C day temperature and heat-treated at 41.6, 49.2, or 55.5° C (average treatment temperatures). Number of particles measured: 45/41.6, 739; 45/49.2, 736; 45/55.5, 665.

Fig. 8. Difference histograms derived from the EF particle-size histograms for the 45° C-grown samples.

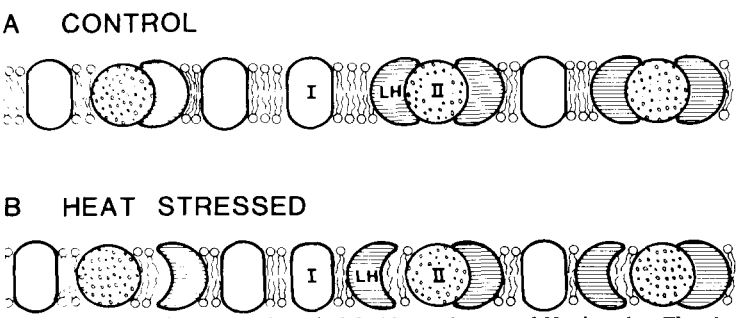

Fig. 9. Model of the chloroplast thylakoid membranes of *N. oleander*. The close association of the photosystem II core complex (II) and the chlorophyll *a/b* light-harvesting complex (LH) that normally exists (A) is lost when the membranes are heat-damaged, resulting in dissociation of the subunits (B). Other complexes present include photosystem I (I).

subunits of dissociated EF particles partition with the PF face or become undetectable as freeze-fracture particles.

Discussion

Analysis of the thylakoid membranes of *N.oleander* by freeze-fracture electron microscopy reveals that morphological alterations of the supramolecular organization of the membranes occur as a result of the pretreatment of the leaves at high temperatures. These alterations, primarily consisting of a decrease in the number of EF particles belonging to the larger (greater than 90 Å) particle-size classes, and a corresponding increase in the number of smaller sized EF particles, are likely to result from a physical dissociation of subunits of the Chl *a/b* light-harvesting complex from the photosystem II core complexes. The similarity in the numbers of particles lost from the larger size categories and gained by the smaller categories (Figs. 6 and 8), and the maintenance of the density of particles on the EF face, suggest that the core complexes are still associated with the EF face. The Chl *a/b* light-harvesting complex presumably lost from these particles must therefore either fracture with the PF face or be otherwise undetectable. The morphological alterations can be correlated to the loss of efficient energy transfer between the pigment complexes as detected by alterations of the chlorophyll fluorescence-emission characteristics [2,3]. These results imply that the heat-induced damage to the photosynthetic apparatus must involve a decrease in the strength of the interactions between the chlorophyll *a/b* light-harvesting complex and the photosystem II core complex (complexes which are normally closely associated; Fig. 9A) to such an extent as to cause a dissociation of the Chl *a/b* light-harvesting complex and the photosystem II core complex (Fig. 9B). The dissociated Chl *a/b* light-harvesting complex may remain in aggregate form, as implied by Fig. 9B, or may dissociate further into monomeric form. Both possibilities are consistent with the observed alteration in energy-transfer efficiency [2,3].

It is evident that the enhanced thermal stability of photosynthesis in the 45° C-grown material is accompanied by a greater stability of the interactions of these integral membrane protein complexes. Whilst it is possible that the proteins themselves are altered in some way by the process of acclimation, the differences in lipid composition and fluidity found in the membranes of *N. oleander* grown under the two thermal regimes [5] may be sufficient to explain the observed differences in membrane heat stability. The association of the protein components with each other in the integral membrane supramolecular complexes, and the interactions between these complexes and membranes lipids, reflect a delicate balance between the hydrophobic and hydrophilic forces that maintain the functional integrity of the membrane. Since these forces are differentially sensitive to temperature (hydrophobic forces enhanced and hydrophilic interactions decreased by increasing temperaturre [13—15]), a membrane system that is optimally balanced to function at one temperature may become unbalanced at another. In the case of the photosynthetic membranes of *N. oleander* used in the present study, it appears that during acclimation to higher temperatures, the environment of the photo-

system II-Chl *a/b* light-harvesting complex aggregate is modified in such a way as to increase the relative strength of the interactions among its subunits, thereby conferring an enhanced stability of the supramolecular complexes at high temperatures.

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