Biochimica et Biophysica Acta, 1099 (1992) 137–144
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BBABIO 43558

Induction of non-bilayer lipid phase separations in chloroplast thylakoid membranes by compatible co-solutes and its relation to the thermal stability of Photosystem II

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(Received 10 July 1991)

Key words: Photosynthesis; Photosystem II; Thermal stability; Chloroplast

High concentrations of compatible co-solutes such as sugars, sugar alcohols and polyols induce the phase separation of non-bilayer forming lipids in thylakoid membrane preparations. Of the co-solutes tested, sucrose, trehalose, betaine and sorbitol all led to extensive phase separation at room temperature. Glucose and glycerol were much less effective. Measurements of the temperature dependence of the fluorescence yield of chlorophyll *a* associated with Photosystem II (PS II), and the relative oxygen evolution efficiency of chloroplasts incubated at elevated temperatures, indicated that the presence of compatible co-solutes led to substantial increases in the threshold temperature for inhibition of PS II-mediated electron transport. The protective effect of the different co-solutes, in order of efficiency, was sucrose > trehalose > sorbitol > betaine > glucose > glycerol. In general, the ability of the different co-solutes to stabilise PS II appeared to parallel their ability to induce non-bilayer lipid phase separation. Comparison of the effects of the co-solutes on isolated chloroplasts with that of heat-stress in unprotected chloroplasts suggest that there is no direct causal link between the phase-separation of non-bilayer lipids from the chloroplast membranes and the increased stability of PS II. It is concluded that the two phenomena are separate reflections of the effects of co-solutes on ordered water at lipid and protein interfaces, respectively.

Introduction

The presence of dry sugars such as trehalose has long been known to have marked effects on the phase properties of lipids [1–3]. Recently, it has been shown that the presence of high concentrations of a wide range of compatible co-solutes can also influence lipid phase behaviour. The addition of sugars, sugar alcohols and polyols have all been shown to lead to marked changes in the relative stability of different lipid phases [4–7]. In particular, the stability of the inverted hexagonal phase (Hex_{II}) of non-bilayer forming lipids is increased at the expense of the lamellar liquid-crystal phase (L_α) resulting in a marked decrease in the temperature of the L_α-Hex_{II} transition at high co-solute concentrations. An increase in the stability of the lamellar gel-phase (L_β), and a corresponding increase in the L_β-L_α transition temperature, is also observed but these effects tend to be less marked.

The chloroplast thylakoid membrane is particularly rich in non-bilayer forming lipids. The non-bilayer lipid monogalactosyldiacylglycerol typically accounts for 40–50% of the membrane lipid fraction [8]. Preliminary measurements in this laboratory [9] showed that the addition of high concentrations of compatible co-solutes leads to extensive non-bilayer phase separations in thylakoid membranes. Earlier studies on heat-stressed chloroplast membranes indicated that phase separation of non-bilayer lipids in such membranes is accompanied by a physical dissociation of the light-harvesting antennae and core particles of Photosystem II (PS II) leading to a destacking of the thylakoid membrane [10–12]. The threshold temperature for the structural changes occurring in heat-stressed chloroplasts was found to be similar to that for the inhibition

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1'-dimethylurea; PS II, Photosystem II; LHC II, light-harvesting chlorophyll *a*/*b* complex; F_o, F_m and F_v minimum, maximum and variable components of the fluorescence yield of PS II; T_i and T_m, threshold temperature and maximal temperature for heat-induced increase in F_o.

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of PS II-mediated electron transport in such membranes [11] suggesting that non-bilayer forming lipids might be involved in the stabilisation of PS II within the thylakoid membrane [13,14].

This study is aimed at investigating the co-solute induced phase separation of non-bilayer lipids in chloroplast thylakoid membranes in more detail and determining the effect of such separations on the organisation and activity of PS II.

Materials and Methods

Chloroplast isolation. Chloroplasts were isolated from the leaves of 2–3-week-old pea seedlings by the method of Stokes and Walker [15]. The isolated chloroplasts were first washed in an assay medium of 50 mM phosphate buffer (pH 7.6), 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2.5 mM EDTA containing 0.4 M sucrose as an osmoticum. They were then resuspended in assay medium containing no osmoticum to disrupt the chloroplasts. Following centrifugation at $3000 \times g$, the chloroplasts were resuspended either in phosphate assay medium (pH 6.0 or pH 7.6), or in Mes assay medium consisting of 40 mM Mes (pH 6.0), 10 mM NaCl, 5 mM $MgCl_2$, containing the appropriate concentration of co-solute.

Steady-state fluorescence measurements. Chlorophyll *a* fluorescence was measured at 685 nm using a modified Perkin Elmer MPF-44A spectrofluorimeter. The fluorescence yield of chloroplasts was measured either under conditions in which all PS II traps were open (F_o) or when all PS II traps were closed (F_m). The temperature dependencies of F_o and F_m were determined both from steady-state measurements and measurements of fluorescence induction.

In the steady-state measurements, the value of F_o was continuously monitored using a weak modulated 430 nm excitation beam and samples containing chloroplasts (~5 μg chl ml^{-1}) together with 2.5 mM $K_3Fe(CN)_6$ and 6.25 mM NH_4Cl as electron acceptor and uncoupler, respectively. Measurements of F_m were made in the presence of 33 μM DCMU with the samples simultaneously illuminated with a broad-band blue unmodulated actinic beam to ensure closure of all PS II traps. In both cases, the samples were heated at a rate of 2 C° min^{-1}.

Fluorescence induction measurements. Measurements were made using a custom-built fluorimeter similar to that described in earlier studies [16]. Aliquots (3 ml) of chloroplasts (5 μg chl/ml) suspended in Mes assay medium (pH 6.0) containing 30 μM DCMU and either 0.4 M or 2.4 M sucrose were placed in the thermostatted cuvette of the fluorimeter. The excitation beam (intensity 20 μmol m^{-2} s^{-1}) was isolated from a quartz-halogen lamp using a broad-band blue Schott filter and Balzer neutral-density filters. Fluorescence was isolated using a Balzer 680 nm interference filter. The photomultiplier signal was digitised at a 5 kHz sampling rate (Datatech 812 A/D converter) and stored on an AT-compatible microcomputer.

Oxygen evolution measurements. Oxygen evolution was measured using a Hansatech oxygen electrode. Aliquots of 2.5 ml of assay medium containing appropriate concentrations of co-solutes were pre-incubated at different temperatures. 10 μl samples of concentrated chloroplast suspension (~1 mg chl ml^{-1}) were added to the pre-heated media and incubated for 5 min. The sample was then rapidly cooled on ice and finally re-equilibrated at 30°C for 5 min prior to measurement at 30°C. Measurements were made under saturating light intensities in the presence of 2.5 mM $K_3Fe(CN)_6$ and 6.25 mM NH_4Cl.

Electron microscopy. Freeze-fracture measurements were made using a Polaron freeze-fracture accessory and the replicas were viewed using a Philips EM301G electron microscope.

Results

Non-bilayer lipid phase separation

Typical freeze-fracture electronmicrographs of chloroplast thylakoids thermally quenched from 25°C in the presence of high concentrations (2.4 M) of sucrose are presented in Fig. 1. The freeze-fracture replicas are characterised by the presence of regions of phase separated non-bilayer forming lipid of the type seen in chloroplast membranes subjected to heat-stress [11,12] or low pH [17]. The phase-separated lipid is usually in the form of three-dimensional blocks of cylindrical micelles. Typical examples are shown in Fig. 1b and c.

Accurate assessment of the extent of phase separation is extremely difficult. The path of the fracture-plane differs for every chloroplast and the nature and relative extent of the different membrane fracture-faces exposed is extremely variable. As a first approximation, the number of phase-separated regions observed in the fracture-planes of 15 chloroplasts chosen at random were counted for each preparation studied. The total number of phase-separated regions per chloroplast, it must be emphasised, is likely to be much higher as only a very limited amount of any given chloroplast lies in the fracture-plane.

Replicas of chloroplasts suspended in assay medium containing 2.4 M sucrose thermally quenched from room temperature (23°C), revealed an average of 2.6 phase-separated regions per chloroplast. Little difference in the frequency of phase separation was observed in increasing the thermal quenching temperature to 50°C and/or lowering the sucrose concentration to 1.5 M sucrose. The frequency of phase-separation decreased sharply at lower sucrose concentrations. No account was taken of the relative areas of the

TABLE I

Frequency of occurrence of regions of phase separated non-bilayer forming lipid in freeze-fractured chloroplasts suspended in media containing different co-solutes

Co-solute	Average number of phase-separated regions in the fracture plane per chloroplast
2.4 M sucrose	2.60
1.8 M trehalose	1.73
3.0 M betaine	3.00
3.0 M sorbitol	1.73
80 wt% glycerol	1.00 [a]
3.0 M glucose	0.80 [a]

[a] Small patches.

different phase-separated regions. In general, however, the size of the phase-separated regions was noticeably larger in samples quenched from higher temperatures and/or higher sucrose concentrations suggesting that increases in phase-separation in such samples was occurring but by the growth of existing phase-separated lipid aggregates rather than by nucleation at new sites.

Data relating to the extent of phase-separation at 25°C induced by different co-solutes is listed in Table I. Precise comparisons are extremely difficult but the data indicate that most phase-separation was seen in the presence of sucrose and trehalose (disaccharides), sorbitol (sugar alcohol) and betaine (amino-sugar). Glycerol (polyol) and glucose (monosaccharide) were much less effective in inducing phase separations. Phase-separation of non-bilayer forming lipids in heat-stressed chloroplasts is normally accompanied by a dissociation of the light-harvesting antennae from the core particle of PS II leading to a loss of granal stacking [11]. In contrast, despite extensive phase-separation, the grana membranes of chloroplasts suspended in 2.4 M sucrose are still in a mainly stacked state. The co-existence of grana stacks and phase-separated non-bilayer lipid within the same chloroplast is clearly demonstrated in the electronmicrograph presented in Fig. 1a. Careful examination of freeze-fracture replicas of this type suggested that while some limited destacking of the grana may occur, extensive destacking of the type seen in heat-stressed chloroplasts definitely does not take place.

Photosystem II activity

The effect of the presence of compatible co-solutes on the stability of PS II was examined by measuring the temperature dependence of the fluorescence yield of chlorophyll *a* associated with PS II. Fluorescence was monitored under conditions in which all PS II traps remain open (F_o) and in which all PS II traps are

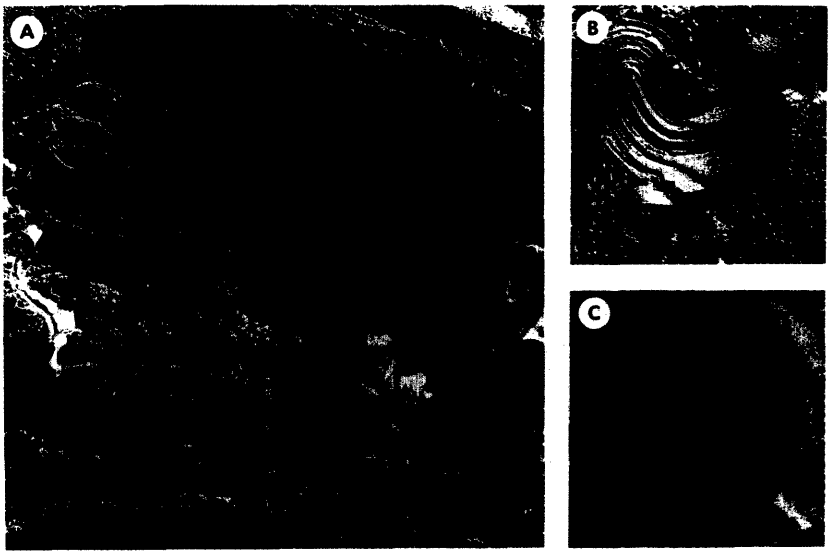

Fig. 1. Typical freeze-fracture electronmicrographs of chloroplast thylakoids, suspended in Mes assay medium (pH 6.0) containing 2.4 M sucrose. Note the presence of cross-fractured grana stacks (GS) co-existing with areas of phase-separated non-bilayer forming lipid (NBL). Scale bar 100 nm.

closed (F_m) as outlined in Materials and Methods. Measurements made using chloroplasts suspended in media containing no sucrose and 2.4 M sucrose are presented in Fig. 2. In both cases, the value of F_o initially increases and then decreases on heating while that of F_m tends to decrease throughout the whole temperature range. The decrease in F_m can be divided into two parts. The decrease occurring between room temperature and about 40°C, as shown by Sundby et al. [19], is largely reversible while that occurring at higher temperatures is almost entirely irreversible.

The main effect of the presence of high concentrations of sucrose is to displace the threshold temperature for the heat-induced increase in F_o, and the corresponding decrease in the variable component of fluorescence ($F_v = F_m - F_o$) associated with PS II-mediated electron transport, to much higher temperatures. The initial value of F_m, and the reversible component of the decrease in F_m, are also somewhat reduced.

In the case of F_o, two characteristic temperatures can be identified T_i, the temperature at which F_o first increases on heating and T_m, the temperature at which it reaches a maximum value and the variable fluorescence F_v is eliminated. Under the measuring conditions used to obtain the plots presented in Fig 2, the presence of 2.4 M sucrose increased the value of T_i from about 28°C to about 56°C and the value of T_m from 48°C to 64°C.

The values of T_i and T_m are both strongly pH dependent. A plot illustrating the variation of T_m with pH for chloroplasts suspended in sucrose-free phosphate and Mes/Hepes buffers is presented in Fig. 3. T_m values were rather higher in the presence of Mes/Hepes buffers than in phosphate buffer but the highest values of T_m were observed in the range pH 6.0–6.5 in both cases. Weiss [20] has reported a similar decrease in the thermal stability of chloroplasts at alkaline pH. The fluorescence measurements described below were carried out at pH 7.6 in phosphate-buffered assay medium but essentially the same results, apart

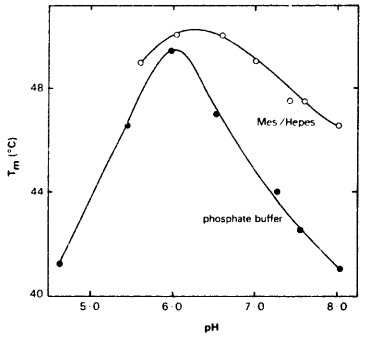

Fig. 3. Plot of T_m as a function of pH for chloroplasts suspended in sucrose-free assay media containing 50 mM phosphate (●) or 40 mM Mes/Hepes (○) as buffer.

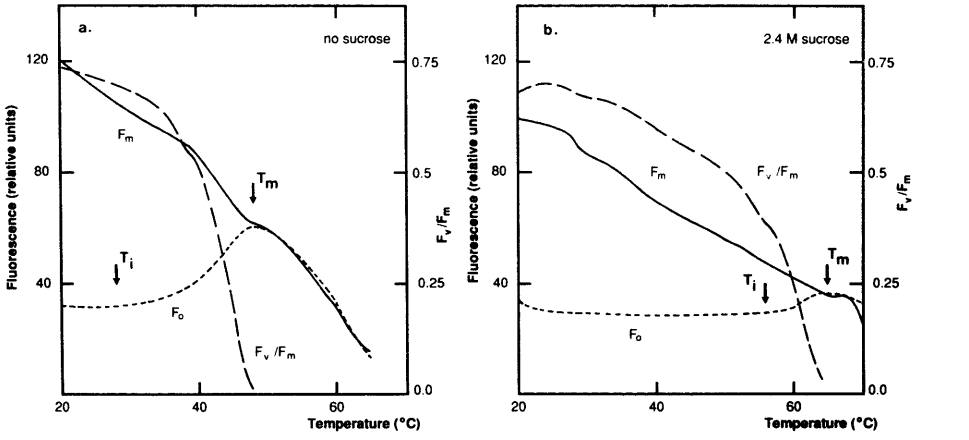

Fig. 2. Plot of F_o, F_m and F_v/F_m as a function of temperature for chloroplasts suspended in (a) sucrose-free phosphate assay medium (pH 6.0) and (b) the same assay medium containing 2.4 M sucrose. Samples were heated at a rate of 2 C° min^{-1}.

from increases in T_i and T_m of the type illustrated in Fig. 3, are obtained if the experiments are repeated at other pH values.

The increase in F_o seen in heat-treated chloroplasts is believed to reflect damage to PS II-mediated electron transport, probably as a consequence of damage to the oxygen evolution apparatus [18,19]. This damage is reflected in the results presented in Fig. 4 showing the effect of incubating chloroplasts for 5 min at different temperatures, in the presence and absence of sucrose, on oxygen evolution efficiency. In the presence of 2.0 M sucrose, the threshold temperature for loss of PS II-mediated oxygen evolution is raised by about 20°C with respect to chloroplasts suspended in 0.4 M sucrose. Values of T_m, obtained from fluorescence measurements made on the same samples, are indicated in the figure for comparison. In both cases, T_m corresponds closely to the temperature for the total loss of oxygen evolution capacity.

The dependence of T_m on the concentration of co-solute in the suspension medium for chloroplasts suspended in media containing sucrose, trehalose, sorbitol, betaine, glucose and glycerol is illustrated in Fig. 5. A similar dependence was observed at rather lower temperatures for T_i (results not shown). The relative effectivity of the different co-solutes in raising the value of T_m is; sucrose > trehalose > sorbitol > betaine > glucose > glycerol. The co-solutes that are most efficient in inducing non-bilayer lipid phase separations in chloroplast membranes are thus also the most efficient in stabilising PS II.

Measurements of the temperature dependence of PS II-associated chlorophyll fluorescence of the type

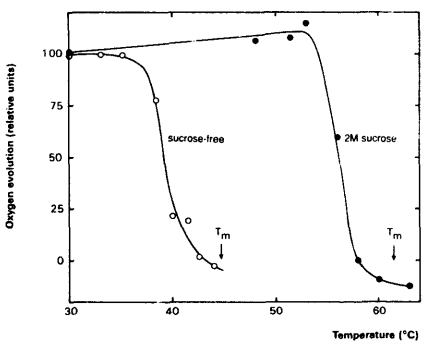

Fig. 4. Plot showing inhibition of oxygen evolution as a function of incubation temperature for chloroplasts suspended in sucrose-free (○) and 2.0 M (●) sucrose phosphate assay medium at pH 7.6. Samples were incubated in pre-heated media for 5 min and oxygen evolution measurements performed at 30°C according to the protocol described in Materials and Methods. Arrows indicate T_m values estimated from fluorescence measurements of the type shown in Fig. 2.

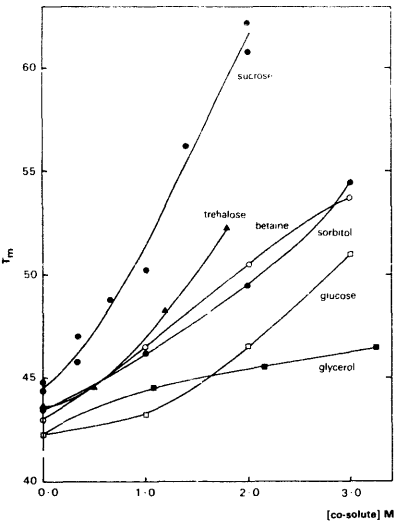

Fig. 5. Plot of T_m as a function of co-solute concentration for chloroplasts suspended in phosphate assay medium containing sucrose (○), trehalose (▲), sorbitol (●), betaine (◇), glycerol (■) and glucose (□) at pH 7.6.

described above are inevitably influenced by the rate of heating of the samples. Damage to PS II, as reflected in losses of the variable component of fluorescence F_v, increases with the length of exposure of the samples to high temperatures [21]. In order to eliminate effects due to accumulated damage associated with slow heating rates, we measured the fluorescence induction curves of samples that had been resuspended, and then incubated for 5 min, in medium pre-heated to different temperatures. Typical fluorescence induction curves obtained for chloroplasts suspended in the presence of 0.4 M and 2.4 M sucrose are presented in Fig. 6. Plots of the temperature dependence of F_v/F_m calculated from these curves are shown in Fig. 7.

Chloroplasts resuspended in high concentrations of sucrose at room temperature are characterised by reduced values of F_v/F_m. This loss is accompanied by a slight reduction in the sigmoidicity of the induction curve. These changes, as discussed in more detail below, probably reflect a limited destacking of the thylakoid membranes in the presence of high sucrose concentrations. The main effect of the higher sucrose concentration, however, is to reduce the loss of F_v normally seen on heating brought about by the irreversible increases in F_o and decreases in F_m. The values of F_v/F_m calculated from the induction curves are in good agreement with the corresponding steady-

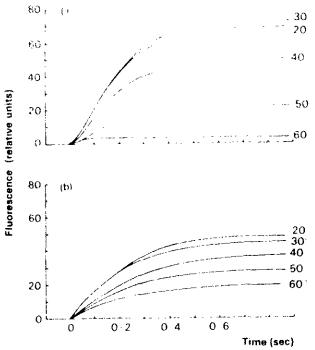

Fig. 6. Typical plots of fluorescence induction curves for DCMU-poisoned chloroplasts suspended in phosphate assay medium (pH 6.0) containing (a) 0.4 M sucrose and (b) 2.4 M sucrose. Data were measured after incubation for 5 min in pre-heated media at the indicated temperatures.

state fluorescence data (shown in Fig. 2) at temperatures below about 40°C, where the irreversible changes are small. At higher temperatures, where the irreversible changes predominate, F_v/F_m decreases much less sharply for the induction curves than for the steady state measurements. This reflects the greater damage experienced by the steady state samples associated with the longer time spent at high temperatures in such measurements. The protective effect of high sucrose concentrations is, nevertheless, quite clear.

Reversibility of co-solute induced changes

The reversibility of the effects of sucrose on the organisation of PS II was tested by first suspending chloroplasts in assay medium containing 2.4 M sucrose, allowing the sample to equilibrate at 20°C for 10 min and then centrifuging the suspension to reconcentrate the chloroplasts prior to resuspension in sucrose-free assay medium. Following resuspension, part of the sample was subjected to freeze-fracture and part used for fluorescence measurements. An aliquot of the original sample, suspended in 2.4 M sucrose, was also put aside for freeze-fracture for comparison. The resuspended sample showed only limited phase-separation; approx. 5–10% of that seen in the presence of 2.4 M sucrose. The temperature dependencies of F_m and F_o were indistinguishable from those seen for fresh suspensions of sucrose-free chloroplasts. Similar results were obtained if the samples were heated to 50°C for 5 min prior to cooling and resuspension in sucrose-free medium.

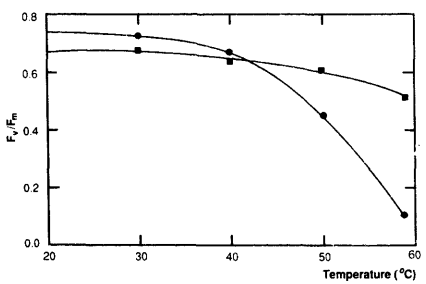

Fig. 7. Plots of the temperature dependencies of values of (a) F_v/F_m and calculated from the fluorescence induction curves presented in Fig. 6. Measurements made in the presence of 0.4 M sucrose (●) and 2.4 M sucrose (■).

Discussion

Addition of sugars, sugar alcohols or polyols to dispersions of pure phosphatidylethanolamines leads to a marked lowering of their bilayer to non-bilayer (L_α–H_{II}) transition temperature [4–7]. Similar changes would be expected to occur in lipid dispersions containing high concentrations of the non-bilayer forming lipid monogalactosyldiacylglycerol and in membranes, such as chloroplast membranes, that contain high concentrations of this lipid. The is confirmed, in the case of the thylakoid membranes of higher plant chloroplasts, by the freeze-fracture measurements reported in this investigation.

Co-solute induced phase-separation of non-bilayer forming lipids in thylakoid membranes leads to the formation of cylindrical inverted lipid micelles (Fig. 1) closely resembling those seen in total lipid extracts of thylakoid membranes [22], heat-stressed chloroplasts [11,12] and chloroplasts exposed to low pH or phospholipase A_2 [17]. The relative efficiency of different co-solutes in inducing phase-separation in pure lipids systems is disaccharides > sugar alcohols > amino sugars > polyols and monosaccharides [7,9]. Measurements of the relative frequency of non-bilayer lipid structures in thylakoids suspended in different co-solutes (Table I) suggest that the relative efficiency of the different co-solutes in inducing phase-separations is similar in the chloroplast system. The extent of phase-separation, as would be anticipated, increases with the concentration of co-solute and temperature.

In the case of heat-stressed chloroplasts, the threshold temperature for phase-separations of this type correlates closely with the threshold temperatures for heat-induced loss of PS II-mediated electron transport and the dissociation of light-harvesting antennae from the core particle of the PS II light-harvesting apparatus [11,12]. One of the main aims of the present study was to determine whether or not similar correlations existed for co-solute induced phase-separation.

The oxygen evolution measurements presented in Fig. 4 clearly indicate that the presence of high concentrations of compatible co-solutes, rather than potentiating damage to PS II mediated electron transport, is extremely effective in protecting PS II against heat-induced damage. This protective effect is reflected in the capacity of these solutes to raise the values of T_i, the threshold temperature for heat-induced increases in F_o, and T_m, the temperature for complete loss of the variable fluorescence F_v (Figs. 2 and 5). The relative order of the different co-solutes in order of their increasing efficiency in protecting PS II is the same as that for their increasing efficiency in promoting the formation of non-bilayer phases.

Sundby et al. [19] have demonstrated that mild heat-stress leads to the dissociation of peripheral light-harvesting chlorophyll *a*/*b* complexes (LHC II) from the core particle of PS II and the subsequent migration of the core particle and its tightly bound LHC II to the stromal membrane. This dissociation would be expected to lead to a reduction in grana stacking [11,12]. Resuspension of chloroplasts in the presence of high sucrose concentrations leads to reductions in F_v and the sigmoidicity of the fluorescence induction curves (Figs. 6 and 7) resembling those reported for chloroplasts subjected to mild heat stress [19]. This could be taken to reflect an increased dissociation of LHC II from the PS II core. Freeze-fracture electronmicrographs of the type shown in Fig. 1a, however, demonstrate clear evidence of stacked grana in such samples. It is possible that a limited dissociation of LHC II is occurring in these samples but extensive destacking of the type associated with non-bilayer lipid phase-separation in heat-stressed chloroplasts is not observed. This would seem to indicate that the non-bilayer lipids are not essential for the preservation of LHC II binding in the presence of high concentrations of sucrose at least.

Our results clearly demonstrate that the presence of high concentrations of co-solutes leads both to the stabilisation of PS II-mediated oxygen evolution and the phase-separation of non-bilayer lipids. This is in direct contrast to the situation in the systems we have previously studied where the occurrence of non-bilayer lipid phase separations appeared to coincide with decreases in PS II stability [11,12,17]. Thus, whilst there appears to be a link between these two phenomena, it is quite clear that this is not a causal one.

The simplest explanation of the changes seen in the presence of the co-solutes is that they exert independent effects on lipid–lipid interactions and protein–protein interactions within the membrane. Co-solutes such as glycerol and sucrose tend to be excluded from the ordered water existing at the interface of lipid bilayers [23] and proteins [24–28]. As a consequence, structures with reduced interfacial areas are stabilised with respect to structures with larger interfacial areas. In the case of non-bilayer forming lipids this leads to a lowering of the transition temperature between bilayer and non-bilayer phases [4–7] and in the case of proteins to an increase in thermal denaturation temperatures [24–28].

The phase-separation of non-bilayer lipids appears to be an example of the first of these phenomena whilst the protection of oxygen evolution appears to be an example of the latter phenomenon. Further evidence indicating that the protection of the oxygen evolution apparatus reflects a stabilisation of the binding of the extrinsic membrane components to the main body of PS II will be presented in a separate paper dealing with the co-solute stabilisation of oxygen-evolving PS II cores (Williams, W.P. and Gounaris, K., unpublished data).

It should be emphasised that the present findings do not necessarily exclude our earlier suggestion that non-bilayer lipids play a role in the packaging PS II complexes within the thylakoid membrane [13,14]. They may, for example, be involved in sealing the PS II units into the thylakoid membrane: a factor important for chloroplast function but one that is not directly reflected in the parameters measured in this study.

References

1 Crowe, J.H., Crowe, L.M. and Chapman D. (1984) Science 223, 701–703.
2 Tsvetkov, T.D., Tsonev, L.I., Tsvetkova, N.M., Koynova, R.D. and Tenchov, B.G. (1989) Cryobiology 26, 162–169.
3 Crowe, L.M. and Crowe, J.H. (1988) Biochim. Biophys. Acta 946, 193–201.
4 Bryszewska, M. and Epand, R.E. (1988) Biochim. Biophys. Acta 943, 485–492.
5 Koynova, R.D., Tenchov, B.G. and Quinn, P.J. (1989) Biochim. Biophys. Acta 980, 377–380.
6 Williams, W.P., Quinn, P.J., Tsonev, L. and Koynova, R.D. (1991) Biochim. Biophys. Acta 1062, 123–132.
7 Sanderson, P.W., Quinn, P.J., Williams, W.P. and Lis, L. (1991) Biochim. Biophys. Acta 1067, 43–50.
8 Douce, R., Holz, R.B. and Benson, A.A. (1973) J. Biol. Chem. 248, 7215–7222.
9 Williams, W.P., Brain, A.P.R., Quinn, P.J. and Sanderson, P.W. (1990) In Plant Lipid Biochemistry, Structure and Utilisation (Quinn, P.J. and Harwood, J., eds.), pp. 62–64, Portland Press, London.
10 Armond, P., Bjorkman, O. and Staehelin, A. (1980) Biochim. Biophys. Acta 601, 433–442.
11 Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1983) FEBS Lett. 153, 47–52.
12 Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1984) Biochim. Biophys. Acta 766, 199–208.
13 Williams, W.P., Gounaris, K. and Quinn, P.J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. III, pp. 123–130, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague.
14 Quinn, P.J. and Williams, W.P. (1985) In Topics in Photosynthesis (Barber, J. and Baker, N.R., eds.) Vol. 6, pp. 1–48, Elsevier, Amsterdam.
15 Stokes, D.M. and Walker, D.A. (1971) Plant Physiol. 48, 163–165.
16 Dominy, P. and Williams, W.P. (1985) FEBS Lett. 179, 321–324.

17 Thomas, P.G., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1985) FEBS Lett. 183, 161–166.
18 Schreiber, U. and Armond, P.A. (1978) Biochim. Biophys. Acta 502, 138–151.
19 Sundby, C., Melis, A., Maenpää, P. and Andersson, B. (1986) Biochim. Biophys. Acta 851, 475–483.
20 Weiss, E. (1982) Planta 154, 41–47.
21 Hugly, S., Kunst, L., Browse, J. and Somerville, C. (1989) Plant Physiol. 90, 1134–1142.
22 Gounaris, K., Sen, A., Brain, A.P.R., Quinn, P.J. and W.P. Williams (1983) Biochim. Biophys. Acta 728, 129–139.
23 Katz, Y. and Diamond, J.M. (1974) J. Membr. Biol. 17, 87–100.
24 Gekko, K. and Timasheff, S.N. (1981) Biochemistry 20, 4667–4676.
25 Gekko, K. and Timasheff, S.N. (1981) Biochemistry 20, 4677–4686.
26 Gekko, K. and Morikawa, T. (1981) J. Biochem. 90, 39–50.
27 Gekko, K. and Morikawa, T. (1981) J. Biochem. 90, 61–77.
28 Lee, J.C. and Timasheff, S.N. (1981) J. Biol. Chem. 256, 7193–7201.