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Stabilisation of PS-II-mediated electron transport in oxygen-evolving PS II core preparations by the addition of compatible co-solutes

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Addition of high concentrations of compatible co-solutes such as sugars, sugar alcohols and polyols has recently been shown to lead to marked increases in the thermal stability of oxygen-evolution in chloroplasts (Williams et al. (1992) Biochim. Biophys. Acta 1099, 137–144). In this paper, a similar stabilisation is demonstrated for oxygen-evolving PS II core preparations. The presence of such co-solutes appears, however, to have no ability to stabilise PS II reaction-centre preparations against heat-induced changes in their absorption spectrum. Nor do they protect electron transport from artificial electron donors in PS II core preparations lacking the extrinsic 33 kDa polypeptide of the oxygen-evolution system. Measurements performed on core preparations retaining the 33 kDa polypeptide but lacking the 17 kDa and 23 kDa polypetides indicate that the co-solutes protect PS-II-mediated electron transport by stabilising the binding of the 33 kDa polypeptide to the core complexes. These findings are discussed in terms of an extension of the general principles underlying the Hofmeister effect observed for soluble proteins to the stabilisation of photosynthetic membrane preparations.

Introduction

Photosystem II (PS II) is generally recognised as being one of the most thermally labile components of the photosynthetic apparatus (see reviews, Refs. 1 and 2). It has been known for some time that the threshold temperature for damage to PS II in thermophilic algae [3–5] and certain chilling-sensitive plants [6–8] can be increased by raising their growth temperature.

In most cases, these increases have been accompanied by parallel increases in the saturation of the fatty acids of the lipids of their photosynthetic membranes. Similar increases in the threshold temperature for PS II damage have been reported for higher plant chloroplasts [9] and algal cells [10] subjected to catalytic hydrogenation and the chloroplasts of a fatty acid desaturase mutant of *Arabidopsis* characterised by reduced amounts of polyunsaturated fatty acids [11]. Observations of this type have been interpreted as indicating that the level of membrane lipid saturation plays a major role in determining PS II stability.

We have recently shown that the addition of compatible co-solutes such as sugars, sugar alcohols and polyols leads to marked increases in the thermal stability of PS II [12,13]. The addition of these solutes, however, was also shown to trigger non-bilayer lipid phase separations in chloroplast membranes, casting doubt on the idea that PS II stabilisation, in this case at least, was associated with membrane lipid effects.

It is well established that the presence of compatible co-solutes stabilises soluble proteins against thermal denaturation [14–21]. Recent studies have shown that they can also have marked effects on membrane lipid stability [22–25]. We suggested, therefore, that the effects of the co-solutes on thylakoid membrane stability and the stabilisation of PS-II-mediated electron transport might be due to independent effects on the lipid bilayer and the photosynthetic apparatus of PS II [13].

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1'-dimethylurea; DPC, diphenyl carbazide; DCPIP, dichlorophenolindophenol; PS II, Photosystem II; Chl *a*, chlorophyll *a*; F_0, F_m and F_v, minimum, maximum and variable fluorescence yields of PS II; T_1 and T_m threshold temperature and maximal temperature for heat-induced increase in F_0.

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In this study, we report the effects of the addition of compatible co-solutes on detergent-stabilised oxygen-evolving PS II core and PS II reaction centre preparations that contain relatively low amounts of lipid. We demonstrate that the degree of PS II stabilisation in oxygen-evolving core preparations is essentially identical to that seen in isolated chloroplasts. Evidence is presented indicating that PS II stabilisation is dependent on the preservation of the binding of the 33 kD extrinsic polypeptide of the oxygen-evolution apparatus to the PS II reaction centre.

Materials and Methods

Isolation of oxygen-evolving cores

Chloroplasts were isolated from the leaves of 2–3-week-old pea seedlings by the method of Stokes and Walker [26]. Oxygen-evolving PS II cores [27] and PS II reaction centres [28] were isolated as described elsewhere. The preparations, suspended in an assay medium consisting of 0.4 M sucrose/10 mM NaCl/5 mM $CaCl_2$/40 mM Mes (pH 6.0) were stored at −65°C. The concentrated suspensions were diluted and resuspended in aliquots of the same medium containing appropriate concentrations of co-solutes as required.

Fluoresence measurements

Chlorophyll *a* fluorescence was measured using a modified Perkin Elmer MPF-44A spectrofluorimeter. Fluorescence was excited using a weak modulated 430 nm excitation beam and detected at 685 nm. Samples (Chl concentration 2–5 $\mu g\ ml^{-1}$) contained 2.5 mM $K_3Fe(CN)_6$ as electron acceptor and 6.25 mM NH_4Cl as uncoupler to ensure that PS II traps remained open during measurements of F_0. 33 μM DCMU and 1.0 mM $NH_2OH \cdot HCl$ were added and the samples illuminated with a broad-band blue unmodulated actinic beam to ensure closure of PS II traps during F_m measurements. The samples were heated at a rate of 2 C° min^{-1} during temperature-dependence measurements.

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 of a concentrated suspension of oxygen-evolving cores were added to the pre-heated media (final Chl concentration 6.9 $\mu g\ ml^{-1}$) and incubated for 5 min. The samples were rapidly cooled on ice and re-equilibrated at 25°C for 5 min; their ability to evolve oxygen was then measured in the presence of 2.5 mM $K_3Fe(CN)_6$ and 6.25 mM NH_4Cl. Measurements were made under saturating light intensities.

PS-II-mediated electron transport

Measurements were carried out using a Perkin-Elmer 557 spectrophotometer equipped with a thermostatic circulator. Actinic light was transmitted through a Schott RG660 filter. The samples contained 5 μg/ml of Chl *a*; 0.5 mM DPC and 0.05 mM DCPIP were used as electron donor and electron acceptor, respectively. The extinction coefficient of DCPIP was taken to be 16 $mM^{-1}\ cm^{-1}$.

Electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out using 13.5% (w/v) acrylamide, 4 M urea gels and the Laemmli buffer system [29]. Samples were loaded at a level of 10 μg Chl *a* per lane. Western blotting was performed as described elsewhere [30]. The antibody to the 33 kDa polypeptide was a gift from Professor Bertil Andersson, University of Stockholm.

Results

The effect of high concentrations of compatible co-solutes on the stability of PS-II-mediated electron transport in oxygen-evolving cores was found to be very similar to that previously reported for chloroplast thylakoid preparations [13]. PS II stability was monitored both by measurements of the temperature-dependence of chlorophyll *a* fluoresence (Fig. 1) and by direct measurements of oxygen-evolution efficiency (Fig. 2).

As illustrated in Fig. 1, the value of F_0 increases as the samples are heated above a threshold temperature, T_i, reaching a maximum at a temperature T_m before decreasing at higher temperatures. The value of F_m, in contrast, remains fairly constant until the temperature of the samples reach 45–50°C, but decreases sharply at higher temperatures. The variable component of PS II fluorescence ($F_v = F_m - F_0$), associated with the photochemical activity of PS II, disappears at temperatures above T_m.

Addition of high concentrations of co-solutes leads to marked increases in the values of T_i and T_m and thus raises the threshold temperature for the loss of F_v. Typical T_m values for cores suspended in high concentrations of a range of different co-solutes are set out in Table I. Of the co-solutes tested, sucrose and trehalose (disaccharides) were the most efficient in stabilising the cores against exposure to elevated temperatures followed by sorbitol (sugar alcohol), betaine (amino-sugar), glycerol (polyol) and glucose (monosaccharide).

The only significant difference between the present results and those obtained for chloroplast thylakoids [13] is that the core preparations lack the initial decrease in F_m seen in chloroplasts below 45°C. This decrease reflects the reversible dissociation of the light-harvesting antennae from the core particle of PS

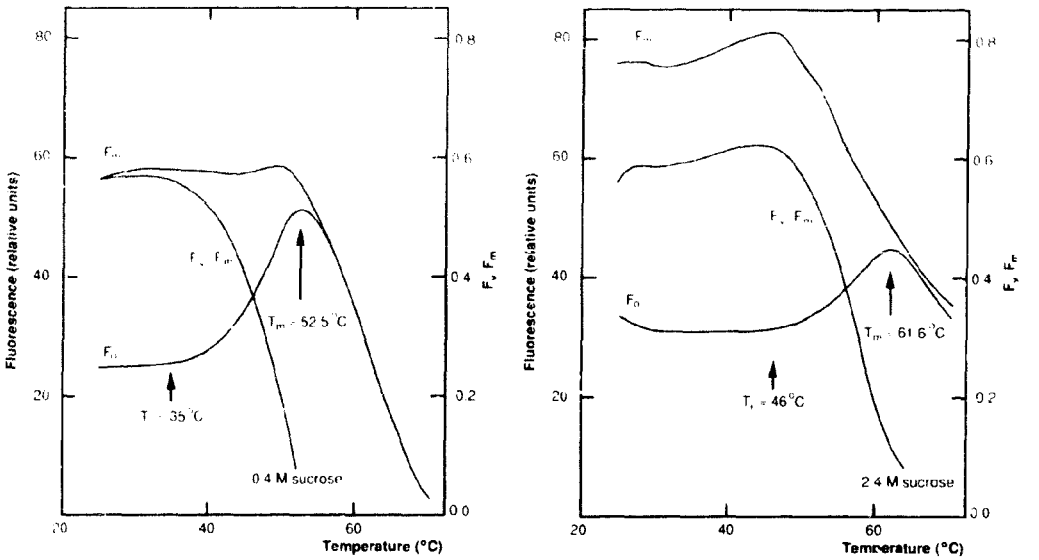

Fig. 1. Temperature-dependence of PS II fluorescence of oxygen-evolving cores suspended in an assay medium containing (a) 0.4 M sucrose and (b) 2.4 M sucrose. Samples contained 2.7 μg Chl ml^{-1}. See Materials and Methods for measurement details.

II [31]. Oxygen-evolving cores lack these antennae complexes and thus show no such decrease.

Typical measurements of the protective effect of sucrose on the oxygen-evolution capacity of PS II cores incubated at elevated temperatures are presented in Fig. 2. The values of T_i and T_m obtained from fluorescence measurements on the same preparations are indicated in the figure for comparison. The presence of high concentrations of sucrose leads to a similar stabilisation of PS-II-mediated oxygen-evolution to that previously reported for chloroplast preparations [13].

In order to determine whether the temperature-dependent lesion leading to the loss of oxygen-evolution activity lies in the reaction-centre of PS II or the oxygen-evolution system, we examined the effect of the co-solutes on the temperature stability of PS II reaction-centre preparations. Incubation of reaction-centre preparations at higher temperatures results in a shift of the red absorption maximum of the centres to shorter wavelengths [32,33]. A plot illustrating the temperature-dependence of this shift is presented in Fig. 3. The presence of co-solutes clearly has no protective effect, as reflected by this test at least, on the reaction centre preparation. This suggests that the protective effect of the co-solutes on the core preparations is

TABLE I

Typical values of T_m for samples of oxygen-evolving cores suspended in assay medium containing different compatible co-solutes

Co-solute concentration (M)	T_m (°C)
No co-solute	48.0
3.25 M glycerol	50.25
3.0 M glucose	50.0
3.0 M betaine	55.0
3.0 M sorbitol	56.0
1.8 M trehalose	58.5
2.4 M sucrose	60.5

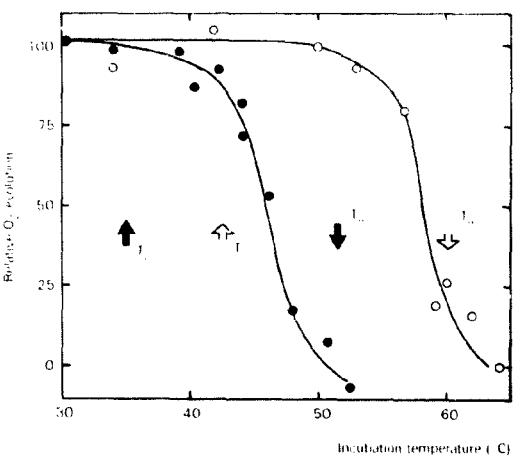

Fig. 2. Oxygen-evolution efficiency of core preparations subjected to 5 min incubation at elevated temperatures plotted as a function of incubation temperature. Samples were incubated in media containing 0.4 M (●) or 2.0 M (○) sucrose. All measurements were made at 25°C under saturating light conditions. T_i and T_m values estimated from fluorescence plots of the type shown in Fig. 2.

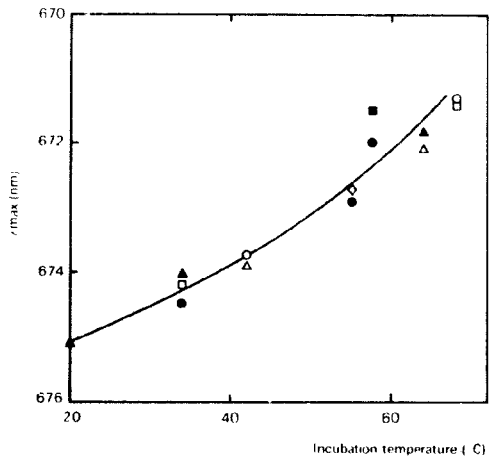

Fig. 3. Plot of shift in absorption maximum of PS II reaction centres incubated in the presence of different co-solutes: △, no co-solute; ○, 3.0 M glucose; □, 80 wt% glycerol; ◇, 3.0 M betaine; ▲, 3.0 M sorbitol; ●, 2.4 M sucrose; and ■, 1.8 M trehalose.

exerted at the level of the oxygen-evolution apparatus rather than that of the PS II reaction centre.

This view is supported by comparison of measurements of the effect of sucrose on the rate of DCPIP-reduction in heat-treated core preparations which contain, or lack, the 33 kDa extrinsic polypeptide of the oxygen-evolution system, presented in Fig. 4. Cores washed with 1.5 M NaCl, which removes the 17 kDa and 23 kDa extrinsic polypetides but leaves the 33 kDa polypeptide, evolve oxygen on illumination and exhibit a similar stabilisation in the presence of high concentration of sucrose to that shown by the original core preparations (Fig. 4a). Cores washed with 1 M $CaCl_2$, which removes all three extrinsic polypeptides [34], do not evolve oxygen under our measuring conditions. Their ability to reduce DCPIP on illumination is restored on the addition of DPC as electron donor, but the ability of high concentrations of sucrose to stabilise PS-II-mediated electron transport against heat-induced damage is not restored (Fig. 4b).

The relationship between compatible co-solute stabilisation of PS-II-mediated electron transport and the binding of the 33 kDa polypeptide was further investigated using SDS-PAGE and Western blotting techniques. Small aliquots (10 μl) of concentrated oxygen-evolving cores pre-washed with 1 M NaCl were added to preheated assay medium (2.5 ml) containing either 0.4 M or 2.4 M sucrose and incubated for 5 min as described above. The samples were then cooled on ice, pelleted by centrifugation and subjected to SDS-PAGE analysis. A typical set of gels and Western blots showing the changing polypeptide composition of the heat-treated cores is presented in Fig. 5.

In the case of the cores suspended in assay medium containing 0.4 M sucrose, there is an essentially complete loss of the 33 kDa polypeptide in samples incubated at temperatures above about 40°C. Little loss of the 33 kDa polypeptide, however, is observed for the same samples suspended in the presence of 2.4 M sucrose even at 60°C. The loss of the 33 kD polypetide is to some extent obscured in the case of the SDS gels by the presence of the D1 and D2 proteins, but is clearly apparent in the corresponding Western blots. The marked stabilisation of the binding of the 33 kDa polypetide by high concentrations of sucrose strongly supports the view that the co-solutes exert their protective effect by stabilising the organisation of the oxygen-evolution apparatus.

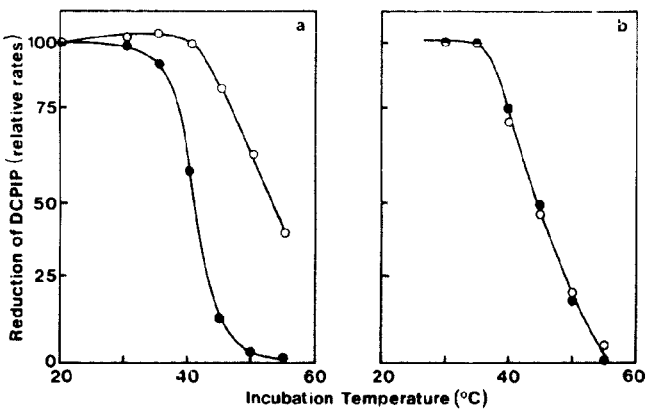

Fig. 4. Plots illustrating the dependence of PS-II-mediated DCPIP reduction on incubation temperature for (a) NaCl-washed cores and (b) $CaCl_2$-washed cores. Samples were incubated in the presence of 0.4 M (●) or 2.0 M (○) sucrose.

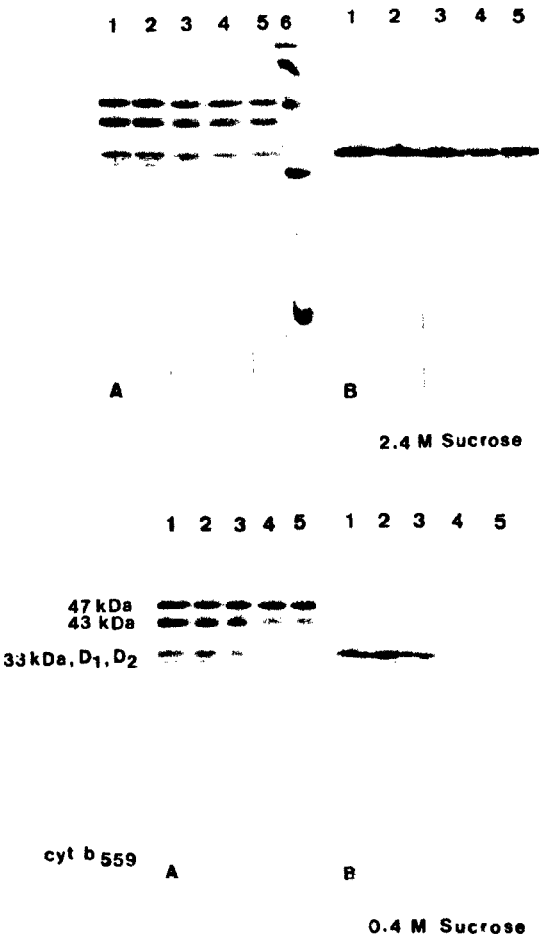

Fig. 5. SDS gels (A), and corresponding Western blots using an antibody to the 33 kDa polypeptide (B), of NaCl-washed core preparations incubated for 5 min at different temperatures in assay medium containing 0.4 M sucrose or 2.4 M sucrose. Samples in lanes 1–5 were incubated at 22, 30, 40, 50 and 60°C, respectively. Lane 6 contains 94, 67, 43, 20.1 and 14.4 kDa molecular mass markers.

Discussion

We have previously reported that high concentrations of compatible co-solutes lead to a marked stabilisation of the oxygen-evolution capabability of thylakoid preparations [12,13]. In this paper, we have shown a similar stabilisation for oxygen-evolving core preparations (Figs. 1 and 2; Table I) by a range of co-solutes. Recently, Papageorgiou et al. [35] have also reported the stabilisation of oxygen-evolving PS II core preparations by betaine against inactivation of oxygen-evolution by high concentrations of NaCl.

It is clear, both from our present study and the study of Papageorgiou et al. [35], that the key factor in this stabilisation is the ability of co-solutes of this type to stabilise the binding of the extrinsic polypeptides associated with the oxygen-evolution system to the PS II core particles. The presence of such solutes appears to have no stabilising effect on the reaction centre complex of PS II against heat-induced changes in their absorption spectrum (Fig. 3). The protective action of the co-solutes thus appears to be mainly, if not exclusively, associated with the stabilisation of the oxygen-evolution apparatus of PS II.

Measurements of PS-II-mediated electron transport from DPC to DCPIP performed on NaCl-washed cores and $CaCl_2$-washed cores point to the key role of the 33 kDa extrinsic polypeptide. Electron transport is only stabilised by high concentrations of compatible co-solutes in cores containing this polypetide (Figs. 4 and 5). It is important, however, to bear in mind that the presence of 5 mM $CaCl_2$ in our assay medium will compensate for any effects specifically associated with the dissociation of the 17 kDa and 23 kDa protein [34]. It is also well established that oxygen-evolution is observed, albeit at a rather lower rate, in core-preparations lacking the 33 kDa polypetide in the presence of high concentrations of NaCl [36]. It is thus probably the role of the extrinsic polypeptides in stabilising other components of the PS II complex, such as the manganese cluster, rather than the presence of the individual polypeptides that is of primary importance.

The ability of compatible co-solutes to stabilise soluble proteins against denaturation is well established and has received considerable attention over many years. Following the work of Timasheff and his collaborators [14–20], it is generally recognised that co-solutes that do not interact with the surface of soluble proteins are normally excluded from the water/protein interface. This depletion which results in an increase in order, and hence a decrease in entropy, of the system is minimised by a reduction in interfacial area. This, in turn, leads to a preferential stabilisation of the folded, or in the case of multi subunit proteins the aggregated, structure over the unfolded state. Co-solutes such as urea and guanadine hydrochloride which exhibit a preferential interaction with non-polar groups in the protein surface, in contrast, tend to favour the unfolded or denatured state.

The general principles involved are equally applicable to any macromolecular assembly. Recently, particular interest has been focused on the effects of salts and compatible co-solutes on the phase behaviour of membrane lipids where the less-chaotropic species tend to stabilise the gel-lamellar and non-bilayer phases (lower surface area/lipid molecule) with respect to the liquid-crystal lamellar phase (higher surface area/lipid molecule) [23–25]. Similar considerations predict that compatible co-solutes should favour the stabilisation of membrane-bound extrinsic proteins.

In the case of chloroplasts, these effects manifest themselves in a number of ways. High concentrations of compatible co-solutes both induce non-bilayer lipid phase transitions and stabilise the oxygen-evolution system against heat-induced damage [13]. The fact that a similar stabilisation occurs in oxygen-evolving core preparations, which contain little lipid, strongly supports the view that these are two related but independent effects. Other manifestations of the same general phenomenon are the ability of high concentrations of co-solutes to minimise freezing damage in chloroplasts [38], and the dissociation of membrane-bound proteins such as the CF_1-ATPase in heat-treated chloroplasts [39].

The ideas outlined above, although implicitly – and occasionally explicitly – recognised, have not been routinely exploited either in the isolation of photosynthetic membrane and/or membrane bound fractions, or in the study of stress conditions. Their application, as exemplified in this study and our related studies on the heat stability of thylakoid preparations [12,13], clearly point to fertile areas for further study.

Acknowledgement

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