Differential Scanning Calorimetry of Chloroplast Membranes: Identification of an Endothermic Transition Associated with the Water-Splitting Complex of Photosystem II†

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ABSTRACT: The structure of spinach thylakoid membranes has been investigated by sensitive differential scanning calorimetry. Six endotherms are observed between 20 and 85 °C, corresponding to order–disorder transitions of different structural domains within the thylakoid membrane. In a medium of relatively high ionic strength, endothermic transitions occur at 42, 54, 65, 72, 79, and 84 °C, with the 65 °C transition being particularly prominent. At a lower ionic strength, transitions are centered at 44, 61, 66, 70, 78, and 83 °C. The 42–44 °C endothermic transition (the A transition) can be correlated with the modification of three electron-transport components or properties associated with photosystem II: (i) release of manganese from the membrane, (ii) the loss of O_2 evolution with water as a donor, and (iii) a decrease in the redox potential of the hydroquinone-reducible cytochrome *b*-559. Both the A transition and the ability to evolve O_2 are irreversibly lost after heating to 49 °C and also after exposure to trypsin, suggesting the involvement of protein in this transition. The interpretation of these observations is that one effect of the A transition involves the thermal disruption of a protein component on the donor side of photosystem II.

Differential scanning calorimetry (DSC)[1] provides a measure of the excess heat capacity of a system as a function of temperature. When applied to aqueous phospholipid dispersions, DSC can yield information on the temperature, enthalpy, and cooperativity of the gel ↔ liquid crystalline phase transitions of these model membranes. By measurement of these properties as a function of selected solution variables, e.g., pH, ionic strength, Ca^{2+} concentration, exogenous protein concentration, etc., considerable insight can be obtained on the behavior of proteins and lipids in biological membranes (Krishnan & Brandts, 1978).

Recently, with the advent of sensitive differential scanning calorimetry, it has been possible to monitor directly the thermotropic properties of individual components within heterogeneous biological membranes (Jackson et al., 1973; Jackson & Sturtevant, 1977). An example is provided by the red cell membrane, in which five distinct transitions are routinely observed, and many of these transitions are sensitive to specific modulators of erythrocyte membrane function (Snow et al., 1978; Low & Brandts, 1978). Through the use of proteases, phospholipases, specific labeling reagents, and modifiers and inhibitors of selected membrane activities, it has been possible to characterize these transitions, and to associate several of the transitions with specific membrane functions (Brandts et al., 1976, 1977, 1978).

The possible applications and advantages of highly sensitive differential scanning calorimetry are, first, that the behavior of a single component within a complex, multicomponent membrane can be studied. Thus, it may be possible to monitor the pH dependence (Brandts et al., 1978) and inhibitor or substrate binding properties (Snow et al., 1978; Low & Brandts, 1978) of an intrinsic membrane protein in situ. Second, in cases where the binding sites of membrane modulators are unknown, it is often possible to identify these sites by observing which transition in the membrane is selectively perturbed upon addition of the modulator. Third, the site of a structural defect in a membrane altered by mutation or disease might be identified through comparison with the calorimetric scan of the wild-type or normal membrane.

Although many of the proteins involved in the function of the energy-transducing membranes have been identified, a calorimetric study of these membranes with sufficient resolution to identify thermotropic transitions of proteins has not been made. In the present work, measurements of the endothermic transitions of chloroplast membranes are presented. The chloroplast thylakoid membrane appears to be a potentially fruitful system in which to apply the calorimetric analysis, as six thermotropic transitions can be detected in the temperature range between 20 and 85 °C. One of these, the A transition in the region 40–50 °C, has been examined in greater detail and identified with the water-splitting function of these membranes.

Experimental Procedures

Chloroplast Preparation and Treatment. Spinach leaves either gathered from a controlled-climate facility maintained at 15 °C on a 10-h light–14-h dark cycle or obtained from a local market were used to isolate chloroplasts according to the procedure of Ort & Izawa (1973), except that the washing medium used was the same as the final suspension medium. The chlorophyll concentration was determined by the method of Arnon (1949). The final suspension medium used for the DSC measurements contained 0.2 M sucrose, 2 mM $MgCl_2$, and either 50 mM potassium phosphate, pH 7.3, and 10 mM

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[1] Abbreviations used: PS II, photosystem II; DSC, differential scanning calorimetry; Hepes, *N*-2-(hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; DMQ, 2,5-dimethyl-*p*-benzoquinone; Tricine, *N*-tris(hydroxymethyl)methylglycine; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

0006-2960/81/0420-0157$01.00/0

KCl (medium I) or 5 mM Hepes–NaOH, pH 7.5 (medium II).

Heat Inactivation Studies. The effect of heat treatment on O_2 evolution and the cytochrome *b*-559 midpoint potential was determined by incubating a small volume (~0.5 mL) of chloroplasts at a concentration of 2–3 mg of chlorophyll/mL in medium II for 3 min in covered test tubes at the temperature specified. The effect of temperature on the aqueous Mn^{2+} signal was determined by similarly heating the chloroplast sample directly in the electron paramagnetic resonance (EPR) cell. Immediately after the 3-min exposure to the elevated temperature, the suspensions were cooled in ice or under cold tap water before assay of electron-transport parameters.

The initial rates of O_2 evolution were determined by using a Clark-type oxygen electrode with chloroplasts diluted to a chlorophyll concentration of 20 μg/mL in a medium consisting of 30 mM Tricine–NaOH, pH 7.8, 0.2 M sucrose, 10 mM KCl, 2 mM $MgCl_2$, and 0.5 mM DMQ. The control rate of oxygen evolution was 175 μmol/(mg of Chl·h) at 20 °C.

The change in midpoint potential of cytochrome *b*-559 after heat treatment was assayed by following the change in amplitude of the reduction by hydroquinone of the ferricyanide-oxidized cytochrome. Cytochrome redox changes were assayed by using the dual-wavelength spectrophotometric apparatus previously described (Cramer & Böhme, 1972), with a wavelength pair at 561 and 570 nm, a 2-nm measuring beam half-bandwidth, and a chlorophyll concentration of 78 μg/mL in a medium consisting of 0.2 M sucrose, 30 mM Tricine–NaOH, pH 7.8, 10 mM KCl, and 2 mM $MgCl_2$.

Manganese release was assayed by monitoring the EPR signal of aqueous Mn^{2+} with a Varian E-109 instrument. Chloroplasts at a concentration of 2.4 mg of Chl/mL were assayed in single scans at room temperature in a 0.25-mm flat cell. The microwave frequency was 9.409 GHz at a microwave power level of 40 mW, a modulation amplitude of 32 G, a receiver gain setting of 1.25×10^4, and a 0.25-s time constant. The amount of manganese detected was determined by comparison with $MnSO_4$ solutions of known concentration.

Trypsin Digestion. The trypsin treatment of chloroplast membranes was conducted with freshly prepared trypsin (Sigma T-0134), and the digestion was terminated by addition of a 2-fold weight excess, relative to trypsin, of soybean trypsin inhibitor (Sigma T-9003).

Calorimetry. Heat capacity measurements were made in a Microcal-1 differential scanning calorimeter (Microcal Inc., Amherst, MA) by using matched 1-mL platinum cells. After the chloroplast suspension was degassed under vacuum, a 1.0-mL aliquot of the suspension at a chlorophyll concentration of 1.8–2.0 mg/mL was added to the sample cell, and an equal volume of buffer was added to the reference cell. The heating rate was 1 °C/min. Enthalpy measurements were obtained by comparison of the integrated area under each peak with the corresponding area derived from a calibrated heat pulse delivered to the reference cell of the calorimeter. The enthalpy of a multilamellar aqueous suspension of lipid vesicles of dipalmitoylphosphatidylcholine was found to be 8.5 kcal/mol with a transition temperature of 41 °C, values very similar to those found in the literature (Melchior & Steim, 1976; Mabrey & Sturtevant, 1976; Lentz et al., 1978).

Results

Typical DSC scans of chloroplast membranes in two media of differing composition and ionic strength are shown in Figures 1 and 2. Six endothermic transitions can be seen between 20 and 85 °C, labeled A–F, in medium I of higher ionic strength containing a high concentration of phosphate

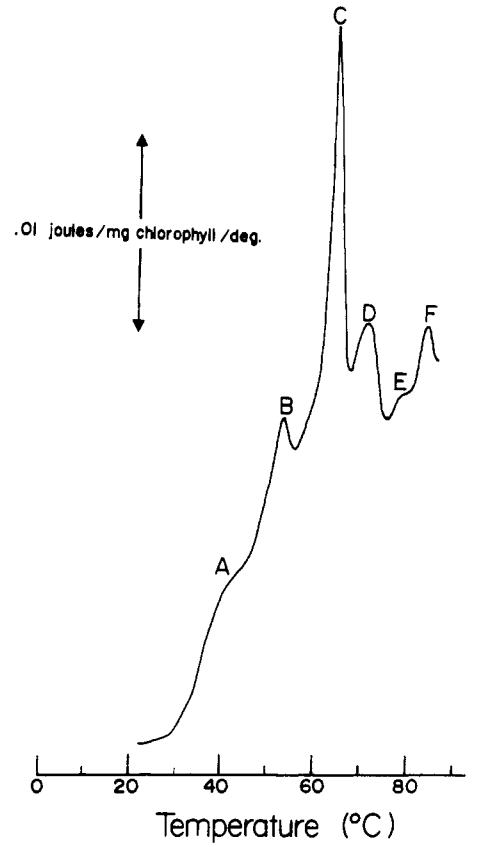

FIGURE 1: Heat capacity of spinach chloroplasts as a function of temperature. The chloroplasts were suspended in 50 mM potassium phosphate, pH 7.3, 10 mM KCl, 2 mM $MgCl_2$, and 0.2 M sucrose at a chlorophyll concentration of 1.8 mg/mL. The six transitions are labeled A–F with the center of the broad A peak at 42 °C, B (54 °C), C (65 °C), D (72 °C), E (79 °C), F (84 °C).

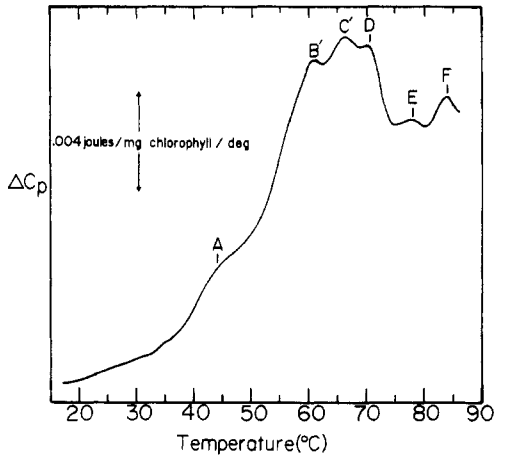

FIGURE 2: Heat capacity of spinach chloroplasts as a function of temperature. The chloroplasts were suspended in 5 mM Hepes–NaOH, pH 7.5, 2 mM $MgCl_2$, and 0.2 M sucrose at a chlorophyll concentration of 1.8 mg/mL. The six transitions are labeled as follows: A (44 °C), B′ (61 °C), C′ (66 °C), D (70 °C), E (78 °C), and F (83 °C). The 61 and 66 °C transitions are labeled B′ and C′ because the 61 °C transition was not observed in Figure 1, and the 65 °C transition of Figure 1 is of much larger amplitude.

(Figure 1). The temperatures (°C) of these transitions, averaged from five different scans, are as follows: for A, 42; B, 54; C, 65; D, 72; E, 79; F, 84. The variability of the peak position measured with different chloroplast preparations was greater for the A transition (±2 °C) than for transitions B–F (±1 °C).

A different calorimetric profile, without the very pronounced C transition, is obtained in medium II of lower ionic strength

in the absence of phosphate (Figure 2). The transitions seen in Figure 2 are labeled A (44 °C), B′ (61 °C), C′ (66 °C), D (70 °C), E (78 °C), and F (83 °C), with the primes indicating that these transitions may have a different origin than B and C of Figure 1. The difference between the conditions used for the calorimetric scans shown in Figures 1 and 2 appears to be one of ionic strength, as replacement of phosphate with bicarbonate, or potassium with sodium, does not substantially alter the DSC scan from that seen in Figure 1. Given the existence of the pattern of discrete transitions shown in Figures 1 and 2, it is of interest to determine whether they can be associated with particular protein and/or lipid components in the membrane. We have concentrated in the present work on the identity of the broad A transition centered, respectively, at 42 and 44 °C in Figures 1 and 2.

An estimate of the area under the A transition in Figure 1 indicates that the enthalpy of this transition is approximately 4.4 mcal/mg of Chl. With the assumption that the chlorophyll/lipid ratio is 1:5 and the lipid/protein ratio is 1:1 (Park, 1966), the specific enthalpy of the A transition would be approximately 0.88 mcal/mg of protein or lipid. This value can be compared with that for an enthalpy measured under our conditions for the dipalmitoylphosphatidylcholine-melting transition at 41 °C of approximately 10 mcal/mg of dry weight. Thus, if the A transition were a lipid-melting transition, it might involve ~10% of the total membrane lipid. On the other hand, protein-unfolding transitions which occur near 42 °C are found to have enthalpies of 4–6 mcal/mg of protein (Privalov & Khechinashvili, 1974). Thus, if A were a protein-unfolding transition, it might represent 15–20% of the membrane protein.

Two observations implicate the involvement of protein in this transition. First, the A transition is irreversible. Thus, chloroplast membranes which have been heated to 49 °C for 5 min prior to the calorimetric scan completely lack the A transition (data not shown). This heat treatment has no effect on transitions B–F. Second, the A transition is gradually removed by trypsin digestion. Figure 3 compares the calorimetric scans of undigested membranes (top) with the scans of mildly digested membranes (middle) or more severely digested membranes (bottom scan). The most obvious effect of the proteolysis is the total disappearance of the A transition and the partial removal of the B and C transitions. Transitions D–F do not appear to be sensitive to trypsin digestion.

Trypsin treatment of chloroplast membranes causes several well-characterized perturbations of chloroplast membrane function. Trypsin digestion inhibits electron transport near photosystem II and modifies the DCMU-binding protein (Regitz & Ohad, 1976; Renger, 1976; Trebst, 1979). Trypsin treatment also causes a negatively directed shift in the midpoint potential of cytochrome *b*-559 (Cox & Bendall, 1972). Trypsin digestion can inhibit the water-splitting reaction at alkaline pH (Selman & Bannister, 1971). In addition, trypsin affects exposed membrane proteins such as ATPase and flavoprotein reductase (Trebst, 1974) and a light harvesting pigment protein (Steinback et al., 1979). The data presented below lead to the conclusion that the removal of the A transition by trypsin is caused at least partly by digestion of a component on the donor side of photosystem II.

The A transition can be associated with the water-splitting proteins or protein–lipid complex of photosystem II through the temperature dependence of changes in electron-transport parameters belonging to system II (Figure 4). Chloroplasts suspended in medium II were exposed to a heat treatment consisting of a 3-min incubation at a series of defined temperatures. O_2 evolution, the redox potential of cytochrome *b*-559, and the aqueous manganese signal were then measured at room temperature or 20 °C as described under Experimental Procedures. The onset of inhibition of O_2 evolution by heat treatment with the class III electron acceptor DMQ (Saha et al., 1971) is near 40 °C, and 50% of the activity is lost after incubation at 44 °C in the same medium used for the DSC scans (open circles, Figure 4). The residual O_2 evolution with H_2O as the donor is approximately 10–20% after incubation at 48 °C (Figure 4).

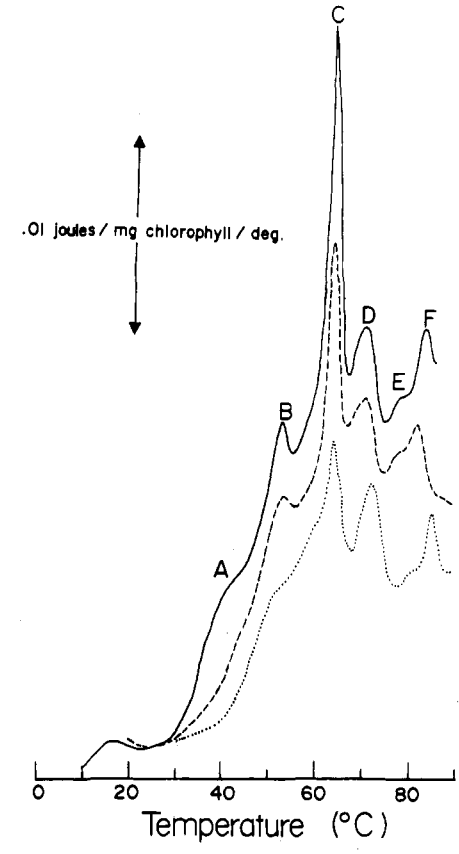

FIGURE 3: Effect of trypsin digestion on the calorimetric transitions of chloroplasts. The DSC spectrum for untreated chloroplasts (top) from Figure 1 is compared with the spectra obtained after exposure of the chloroplasts to trypsin at two levels of effectiveness (two lower scans). Middle scan: Trypsin treatment resulting in inhibition of O_2 evolution by 90%. Chloroplasts were incubated at 100 μg/mL chlorophyll for 5 min with 260 μg/mL trypsin in 50 mM potassium phosphate, pH 7.3, 10 mM KCl, 2 mM $MgCl_2$, and 0.2 M sucrose while the mixture was stirred at room temperature. Then trypsin inhibitor was added at 520 μg/mL for 3 min, and the chloroplasts were washed and resuspended at 1.8 mg of Chl/mL in this same phosphate medium (medium I, see Experimental Procedures). After this trypsin treatment, the residual level of O_2 evolution, assayed as described under Experimental Procedures with methyl viologen as electron acceptor and with 2 μM gramicidin, was 11% of the control level [140 μmol/(mg of Chl·h) at 18 °C]. Bottom scan: Treatment with 100 times the trypsin concentration required to inhibit O_2 evolution by 90%. Chloroplasts were treated with a high concentration of trypsin under conditions of lower ionic strength. Chloroplasts at 50 μg/mL were incubated in 0.1 M sucrose and 5 mM Hepes–NaOH, pH 7.8, for 10 min at room temperature with stirring in the presence of 100 μg/mL trypsin before addition of a 2-fold excess of trypsin inhibitor, centrifugation, and washing. Under these conditions of ionic strength, addition of only 1 μg/mL trypsin caused inhibition of oxygen evolution to 11% of the control level [177 μmol of O_2/(mg of Chl·h)]. Chlorophyll concentration was 1.7 mg of Chl/mL.

Cytochrome *b*-559 is an electron-transport component structurally linked to photosystem II (Cramer & Whitmarsh, 1977). Between one and two molecules of this cytochrome per reaction center are found to be in a relatively high-potential state in which the oxidized cytochrome can be reduced by

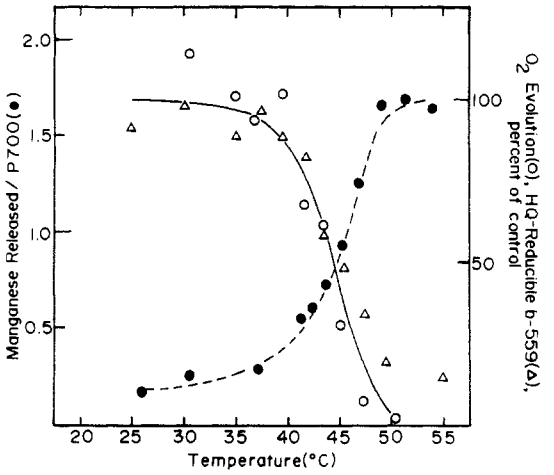

FIGURE 4: Effect of a defined heat treatment on photosynthetic oxygen evolution (○), the amount of high-potential cytochrome *b*-559 (△), and the concentration of aqueous manganese (●). Oxygen evolution was measured as described under Experimental Procedures. High-potential cytochrome *b*-559 was defined by its reducibility by hydroquinone. The absorbance change was measured in a dual-wavelength spectrophotometer as discussed under Experimental Procedures, and 1 mM hydroquinone was added to reduce the cytochrome after chemical oxidation with 0.5 mM potassium ferricyanide. The stoichiometry of the cytochrome reduced by hydroquinone in the control was 2.9 nmol/mg of Chl, using the spectra and extinction coefficients given in Cramer & Whitmarsh (1977). The aqueous manganese signal is plotted as the amplitude of the first derivative signal obtained from the EPR spectrometer, measured for the next to highest field Mn line, between the trough measured at 3493 G and the peak at 3518 G. The concentration of aqueous manganese measured after incubation for 3 min at 55 °C corresponded to approximately 2 Mn per 650 chlorophylls (2 Mn/P700).

ferrocyanide or hydroquinone. Different chemical and physical treatments inhibiting O_2 evolution, including heat (Wada & Arnon, 1971; Horton et al., 1978), trypsin (Cox & Bendall, 1972), and incubation in hydroxylamine (Horton & Croze, 1977), are known to cause a decrease in the level of high-potential cytochrome *b*-559. In the present case, there is a good correlation between the loss of O_2 evolution and the decrease in content of hydroquinone-reducible cytochrome *b*-559. Half the amplitude of reduction of the cytochrome by hydroquinone is lost between 42 and 44 °C (triangles in Figure 4). The 15% residual absorbance change present after incubation at 50–55 °C is attributed to the absorbance change of cytochrome *f* at 561 nm.

Manganese is known to be an essential component for photosystem II activity (Cheniae, 1970). Manganese in solution can be assayed by the characteristic six-band EPR spectrum diagnostic for manganese in an aqueous environment (Garrett & Morgan, 1966). An increase in the aqueous manganese EPR signal in *Chlorella* has been observed between 55 and 60 °C (Donnat & Briantais, 1967). Heating of chloroplasts results in manganese extrusion (Cheniae & Martin, 1970; Wydrzynski & Sauer, 1980). In the present case, there is a close correlation between the fractional increase in the amount of manganese extruded from the membrane (filled circles in Figure 4) and the loss of photosystem II activity. The manganese signal rises suddenly at about 40 °C to a level which remains approximately constant for incubations above 48 °C. The amount of manganese detected at the higher temperatures with medium II corresponds to approximately 2 Mn, and perhaps as many as 3 Mn (data not shown), per P700, using chloroplasts with approximately 1 P700 per 650 chlorophylls (Whitmarsh & Cramer, 1979). The nature of the chloroplast suspension medium strongly affected the amount of EPR-detectable manganese. The level of observable manganese was decreased by a factor of about 20 when medium I containing phosphate was used, presumably because of the formation of manganese phosphate ($MnHPO_4$), whose solubility product is 10^{-13} M^2 L^2 (Sillén & Martell, 1971). The amount of manganese shown in Figure 4 could be increased by a factor of 1.5–2.0 if 10 mM, instead of 2 mM, $MgCl_2$ was used in the suspension medium.

Characterization of the A transition was also further pursued through a study of the effect of specific inhibitors of photosystem II function. Hydroxylamine, DCMU, and Tris treatment all appeared to displace or remove the A transition (data not shown). However, because of the small amplitude of the transition, we could not unequivocally characterize these effects.

Discussion

It is known that photosystem II function is labile toward heat treatment and that it can be inactivated in spinach chloroplasts between 35 and 50 °C (Rumberg et al., 1965; Yamashita & Butler, 1968; Döring et al., 1969; Cheniae & Martin, 1970; Wydrzynski & Sauer, 1980), depending upon the incubation time and perhaps other experimental conditions. The present work describes the magnitude of the enthalpy change and the temperature profile associated with the thermal inactivation. The thermal inactivation could affect one or more sites in the donor–acceptor complex of PS II. The removal of the A transition by trypsin implies that it is associated with a complex in the membrane that is at least partly proteinaceous, with no information available yet on the involvement of lipid. Trypsin can affect at least two or three sites in photosystem II, the DCMU-binding secondary acceptor protein (Regitz & Ohad, 1976; Renger, 1976; Trebst, 1979) and a site on the donor side of photosystem II close to the water-splitting reactions (Selman & Bannister, 1971), as well as cytochrome *b*-559 (Cox & Bendall, 1972). The ability of electron donors to partially restore $NADP^+$ reduction to heat-treated (50 °C) chloroplasts (Yamashita & Butler, 1968) implies that the electron-acceptor side of photosystem II is not as heat sensitive as the donor side and that one site of heat inactivation associated with the A transition involves a protein component on the donor side of photosystem II. The similar temperature profiles of manganese extrusion from PS II, inactivation of O_2 evolution, and the A transition further suggest that Mn extrusion and/or denaturation of the Mn binding protein is a specific molecular event associated with the A transition.

The shift of the cytochrome *b*-559 midpoint potential caused by heating to 40–50 °C suggests that this cytochrome could be another protein in photosystem II that is affected in the A transition. However, an alternate explanation of the shift in midpoint potential, suggested by the very similar temperature profiles of the Mn extrusion and the cytochrome change, is that the latter occurs as a secondary response to the release of manganese (Cramer & Horton, 1975; Horton & Croze, 1977).

The identity of the C transition of Figure 1 will be the subject of future work. From the temperature profile of the heat activation of chloroplast ATPase (Farron & Racker, 1970), it was anticipated that there would be an endothermic transition of the ATPase in this temperature region. However, the large increase in amplitude of the C transition with increasing ionic strength may also be a consequence of increased adsorption of relatively loosely bound protein. Ribulose-1,5-bisphosphate carboxylase is an abundant stromal protein that may adsorb to the thylakoid membranes (Carrillo et al., 1980). Preliminary work indicates that it may be difficult to dis-

criminate between ATPase and carboxylase on the basis of calorimetry alone, as purified CF1 and carboxylase both show an endothermic transition in the region of the C transition (in collaboration with B. R. Selman and W. Widger).

There is one transition sometimes observable in the DSC scans that has not yet been discussed, a small endotherm between 15 and 20 °C. Such an endotherm has been observed by others in chloroplast lipid extracts (Jursinic & Govindjee, 1977). Because it was not present in all of our scans, we have not shown this transition. We mention it because a transition in chloroplast membrane structure or lipid phase in this temperature region has been inferred from Arrhenius plots of photosynthetic electron transport and phosphorylation (Kraayenhof et al., 1971; McEvoy & Lynn, 1972; Gräber & Witt, 1974; Yamamoto & Nishimura, 1976; Nolan & Smillie, 1976, 1977). Further work is needed to determine whether there is an endotherm in chloroplast membranes in this temperature range.

Added in Proof

After this paper was in press, two papers on temperature effects associated with PS II came to our attention: (1) Maison & Lavorel (1977), in which the miss coefficient for O_2 evolution in *Chlorella* shows a maximum at 35–40 °C; (2) Armond et al. (1980), in which heat alterations at 40–55 °C of chloroplast membranes of *N. oleander* are associated with dissociation of the chlorophyll a/b protein complex from the PS II core.

Acknowledgments

We thank Mona Imler and Barbara Freeland for their help with this manuscript, Professor A. Winfree for providing the software for the digitization and integration of the manganese signal, Professor Bruce Selman for helpful discussion, Mark Girvin for assistance with some experiments, and Jack Barnes for technical assistance.

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Fluorospectroscopic Studies of Various Ganglioside and Ganglioside–Lecithin Dispersions. Steady-State and Time-Resolved Fluorescence Measurements with 1,6-Diphenyl-1,3,5-hexatriene†

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ABSTRACT: Molecular motions of 1,6-diphenyl-1,3,5-hexatriene (DPH) in gangliosides (GM3, GM2, GM1, GD1a, and GD1b), GA1 glycosphingolipid, and dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC)–ganglioside mixed dispersions were studied by using techniques of steady-state and nanosecond time resolved fluorescence measurements in the temperature range of 20–50 °C. The total fluorescence decay $s(t)$ was approximated to a best-fit curve of double-exponential decays, and two fluorescence lifetimes were obtained. The values of the shorter fluorescence lifetime in dispersions composed of a single glycosphingolipid component approached those of the longer one on addition of DPPC. The molecular arrangement or microheterogeneity of the hydrocarbon region surrounding DPH molecules changed depending on the ratio of DPPC to ganglioside molecules and on the temperature. The steady-state anisotropy r_s in dispersions composed of a single glycosphingolipid component exhibited smooth changes, not abrupt ones, in the temperature range, in contrast to that in DPPC liposomes. In the various glycosphingolipid dispersions studied, the motion of DPH molecules was the most restricted in the GA1 dispersion. Sialic acid linked to the neutral sugar backbone influenced the hydrophobic region and increased the motion of DPH molecules. In the gangliosides tested, the motion of DPH molecules in the hydrophobic region of GM1 ganglioside was found to be the most restricted. These comparative studies indicate that the ultimate and/or penultimate carbohydrate moieties of the neutral sugar backbone of gangliosides and the topographical difference in the locations of the sialic acid linkage influence the integrity of the membranes including the hydrophobic region.

Glycophingolipids are known to be present in especially high concentration in plasma membranes (Dod & Gray, 1968; Klenk & Choppin, 1970; Weinstein et al., 1970; Gahmberg, 1971; Keenan et al., 1972; Yogeeswaran et al., 1972). This characteristic, together with the great structural variety of their hydrophilic carbohydrate portion, makes them ideally suited for participation in diversified cell surface recognition functions, such as hormonal and immunological regulatory mechanisms of cellular activities, cell growth, cell behavior, and cell to cell interactions [Lee et al., 1976, 1977; Mullin et al., 1976; Besancon et al., 1976; Vengris et al., 1976; Holmgren et al., 1973; King & van Heyningen, 1973; Pierce, 1973; Cuatrecases, 1973; van Heyningen & Mellanby, 1959; Helting et al., 1977; Yogeeswaran & Hakomori, 1975; cf. Hakomori (1975); Yamakawa & Nagai, 1978]. In spite of increasing attention to their significance, our knowledge of their physical properties as membrane forming constituents is still poor compared with that of phospholipids. Only a few glycosphingolipids, such as galactocerebroside (Clowes et al., 1971; Abrahamsson et al., 1972; Oldani et al., 1975; Sharom et al., 1976), globosides (Tinker et al., 1976), and ganglioside mixtures (Gammack, 1963; Hill & Lester, 1972; Abramson et al., 1972; Curatolo et al., 1977; Sillerud et al., 1978; Formisano et al., 1979), have been studied. One reason for this is the difficulty in obtaining sufficient amounts of highly purified compounds for studies. However, we recently developed a procedure for isolating and purifying gangliosides in sufficient amounts for such studies (Momoi et al., 1976; Iwamori & Nagai, 1978). This prompted us to investigate their physicochemical properties. Fluorospectroscopy has been useful for investigating the physical properties of the hydrocarbon region of the membranes through the motion of fluorescent probe molecules (Faucon & Lussan, 1973; Cogan et al., 1973; Shinitzky & Inbar, 1974, Shinitzky & Barenholz, 1974; Galla & Sackmann, 1975; Lentz et al., 1976a,b; Kawato et al., 1977; Dale et al., 1977; Martin & Foyt, 1978). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was used as a fluorophore, and the motions of this compound embedded in vesicular lipid membranes consisting of either various ganglioside molecular species or ganglioside–DPPC[1] mixtures were investigated. Steady-state fluorescence measurements give information on the average motion of the fluorophore, while time-resolved fluorescence measurement provides much information on the fluorescence lifetime and the motion of the fluorophore.

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[1] Abbreviations used: DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoyl-*sn*-glycero-3-phosphorylcholine; DEAE, diethylaminoethyl; ESR, electron spin resonance; Tris, tris(hydroxymethyl)aminomethane. The ganglioside designation conforms to the nomenclature of Svennerholm (1964). The structures of glycosphingolipids used in this work are shown in Table I.

0006-2960/81/0420-0162$01.00/0