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THYLAKOID CONFORMATIONAL CHANGES ACCOMPANYING MEMBRANE PROTEIN PHOSPHORYLATION

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The effect of reversible membrane phosphorylation on the room temperature linear dichroism signal of magneto-oriented pea thylakoids was investigated. Membrane phosphorylation, induced by photoreduction of the plastoquinone pool, resulted in a change in the linear dichroism signal in the region of the red absorption band of chlorophyll. The optical change was due to modifications in selective polarized light scattering which have been shown to be indicative of alterations in the degree of membrane stacking. No changes in linear dichroism due to a reorientation of pigments were observed. It is concluded that phosphorylation of the membrane results in about a 10% destacking of the thylakoids and that this conformational change is implicated in energy redistribution between the two photosystems.

During oxygen-evolving photosynthesis it is now generally recognized that at sub-saturating light intensities the distribution of excitation energy between the two photosystems is regulated. This has been demonstrated by monitoring the fluorescence emission from the two photosystems following the induction of light States 1 and 2 [1,2] or changing the concentration of cations in the thylakoid milieu [3]. Recently it has been shown that the phosphorylation of two surface peptides of the light-harvesting complex (LHC) by a membrane kinase [4] results in a redistribution of energy that is similar to State 2 [5]. This activity is dependent on the reduction of the plastoquinone (PQ) pool and then PS I is favored [6,7]. A membrane phosphoprotein phosphatase reverses the condition to State 1 by removal of the phosphate groups from the LHC peptides [8].

Previous work from this laboratory [9] has been directed towards an experimental verification of membrane conformational changes that might accompany the light state transitions and those following the addition of cations. Such structural changes were first postulated by Bonaventura and Myers [1] and Murata [2] and advanced on theoretical grounds by Seely [10]. We have reported that the addition of divalent cations to magneto-oriented thylakoids results in a very small change in linear dichroism signal due to the LHC superimposed upon a large change in selective polarized light scattering due to membrane stacking [9]. The present report extends this experimental strategy to include similar observations on oriented thylakoids accompanying the State 2 conversion via the PQH_2-induced membrane phosphorylation. No changes in linear dichroism indicative of a reorientation of specific pigments were discernible, but small changes in selective polarized light scattering were readily observed. Comparison of the data with that reported in Ref. 9 suggests that the membrane phosphorylation results in a small decrease in thylakoid stacking.

Envelope-free thylakoids from peas were isolated, washed and stored as described by Bennett et al. [5], and their reaction conditions for thylakoid phosphorylation were used without modification. The

Abbreviations: LHC, light-harvesting complex; PQ, plastoquinone; PS, Photosystem; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

kinase-induced changes in thylakoid energy distribution were monitored by fluorescence using an Aminco-Bowman Spectrofluorimeter. Linear dichroism measurements were made at room temperature on thylakoids oriented in a magnetic field using instrumentation described previously [9].

Fig. 1 shows representative data that illustrate the effect of membrane phosphorylation on the linear dichroism signal ($A_{\parallel} - A_{\perp} = \Delta A$) in the red-wavelength region of thylakoid absorption. Incubation of the thylakoids with ATP in 640 nm light results in a reproducible change in the signal that is characterized by a decrease in ΔA at wavelengths shorter than the peak dichroism at 682–684 nm and a slight increase at longer wavelengths. The difference between the two ΔA spectra is shown in the upper trace of Fig. 2. The difference, $\Delta(A_{\parallel} - A_{\perp})$, which is amplified 5 times for clarity, is a simple sigmoid curve that is very similar to the change in selective polarized light scattering of oriented thylakoids that is brought about by the addition of divalent cations. The magnitude of the ATP-induced change shown here, however, is much smaller and in the opposite direction.

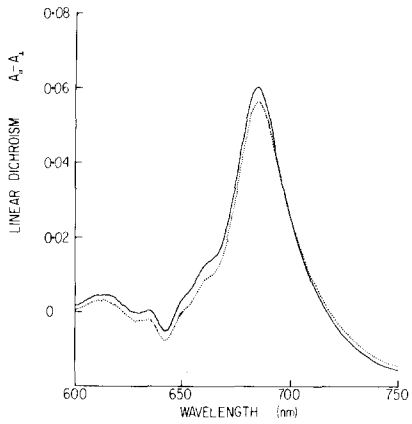

Fig. 1. Room-temperature linear dichroism spectra of thylakoids before (———) and after membrane phosphorylation (· · · · · ·). The thylakoids were suspended in 0.1 M sorbitol/10 mM tricine (pH 8.0)/10 mM $MgCl_2$/1 mM KCl, 5 mM mercaptoethanol/150 μM ATP. A magnetic field of 18 kG was used for orientation of the thylakoids which had an absorbance of 0.7 at 675 nm. After recording the control spectrum (———), the sample was illuminated with light of 640 nm (Baird Atomic Interference Filter plus Corning color glass 2.60) and of an intensity of $5 \cdot 10^3$ erg/cm^2 per s to reduce the plastoquinone and activate the kinase. The spectrum was re-recorded after 5 min illumination (· · · · · ·).

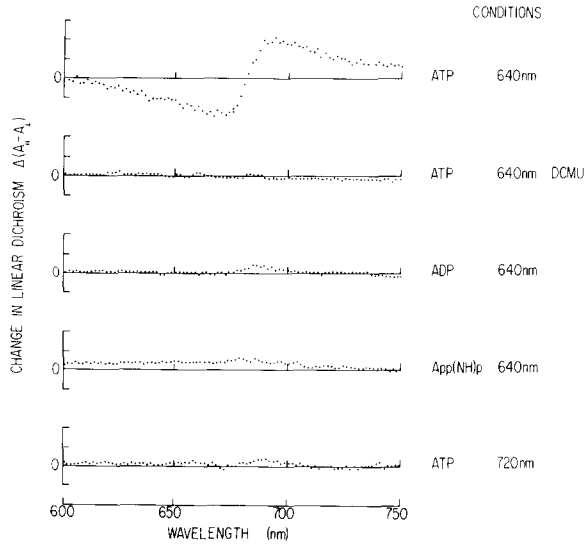

Fig. 2. Changes in linear dichroism signal following illumination of thylakoids under various conditions. Conditions as in Fig. 1. ATP, ADP and App(NH)p were 150 μM and DCMU 10 μM. The 720 nm light (Baird Atomic Interference Filter plus Corning color glass 2.64) was of an intensity of $8.5 \cdot 10^3$ erg/cm^2 per s. Each division on the ordinates is equivalent to 0.001 A.

Fig. 2 also shows the results of several control experiments that are important in the protocol and definitely establish that the effects here are those due to membrane phosphorylation. No change in linear dichroism signal was noted following treatment of thylakoids in conditions that do not result in the reduction of PQ; namely, 640 nm illumination in the presence of DCMU or illumination with 720 nm light. A very small change in ΔA was routinely observed following 640 nm illumination in the presence of ADP but not in the case of the nonmetabolizable analog of ATP, App[NH]p.

It was not experimentally feasible to conduct similar measurements that involved the chemical reduction of PQ by either dithionite or NADPH plus ferredoxin, because the orientation of the samples in the magnetic field precluded mixing once the control ΔA spectrum had been established for a given experimental condition.

The dark reversibility of the effect was studied, and about 60% reversibility was observed in 10 min. Full reversibility was never observed, but the measurement was difficult to conduct at longer time peri-

ods due to base line shifts originating from settling of the sample in the optical path. The partial reversibility was, however, prevented by the inclusion of 10 mM NaF in the reaction medium, which has been shown to inhibit the thylakoid phosphoprotein phosphatase [8].

As mentioned above, the change in linear dichroism signal accompanying the phosphorylation of the thylakoids (Fig. 1 and Fig. 2, upper trace) is similar to the cation induced change in selective polarized scattering reported previously [9]. In that study it was shown that a substantial fraction of the polarized scattering could be eliminated by changing the refractive index of the medium with glycerol. Fig. 3 shows that inclusion of 50% glycerol in the medium eliminated the change in linear dichroism due to membrane phosphorylation suggesting that the ATP-induced changes in linear dichroism signal in Fig. 1 and 2 were scattering changes. However, the elimination of a change in signal in Fig. 3 could also have been due to inhibition of the kinase by glycerol. This possibility was ruled out by following the ATP-photo-induced rate of quenching of chlorophyll fluorescence at room temperature [5,6] to measure the kinase activity. The half-time for the decrease in 680 nm emission was 231 s for conditions similar to those in Fig. 1 and in the presence of glycerol was not significantly different (243 s). These values are in the same range as that found for the rate of change in linear dichroism signal (264 s) and those previously reported for $^{32}P_i$ incorporation in vitro [5] and for the State 1 and State 2 transition in vivo [1,2]. This suggests that the optical changes reported here are in all probability indicative of a membrane conformational change associated with the same physiological event.

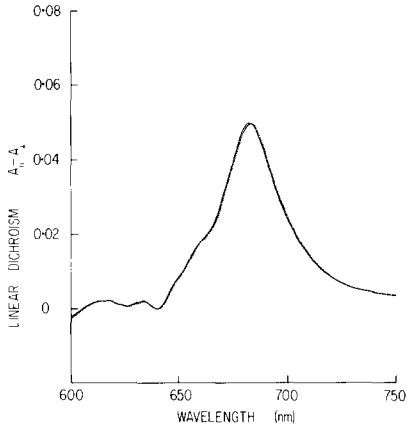

Fig. 3. Room temperature linear dichroism spectra of pea thylakoids before (———) and after membrane phosphorylation (· · · · · ·). The experimental protocol was as in Fig. 1, but the medium was supplemented with 50% glycerol (v/v).

The optical changes are interpreted as a change in the selective polarized light scattering of the thylakoids which has been shown previously to be indicative of the extent of membrane stacking [9]. The scattering change reported here is opposite in direction to that caused by the addition of divalent cations to thylakoids, indicating that the membrane phosphorylation leads to a partial destacking of the thylakoids. The magnitude of the destacking is about 10% of the divalent cation-induced change, and, if the ΔA signal is a quantitative measure of this structural change, it represents either an extremely small change in appression of the entire grana areas or a larger change at some specific sites, such as the periphery of the grana stacks as suggested by Barber [11]. In a study of intact *Chlamydomonas reinhardi* by electron microscopy, Bennoun and Jupin [12] reported a difference in membrane stacking between cells adapted to State 1 and State 2. This work is in contrast to the observations of Vernotte et al. [13] who used similar technology but reported no structural difference between the chloroplasts of preilluminated intact pea levels.

The changes in ΔA reported here are small but reproducible and could have arisen by either a decrease in $A_{\parallel}$, an increase in $A_{\perp}$ or both. It was not possible to detect any ATP-induced changes with certainty in the absolute absorption spectra, $A_{\parallel}$ or $A_{\perp}$, of oriented thylakoids using polarized measuring beams and the increased sensitivity of the difference $A_{\parallel} - A_{\perp}$ was found to be essential. If any reorientation of pigment accompanied the destacking process, it was beyond resolution. At present it is possible to conclude that phosphorylation of the thylakoids by the membrane phosphoprotein kinase results in a conformational change that involves a small change in proximity between the planes of the membranes.

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