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EFFECT OF CATIONS ON THE LINEAR DICHROISM AND SELECTIVE POLARIZED LIGHT SCATTERING OF THYLAKOIDS

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Summary

The effect of cations on the linear dichroism (LD) and selective polarized light scattering of higher plant thylakoids was investigated. The results show that the major change in LD signal caused by the addition of cations is due to a scattering contribution most probably resulting from thylakoid stacking. However, minor changes in the LD signal also occur on the short wavelength side of the main LD band that persist even when a large proportion of the scattering change is eliminated by increasing the refractive index of the medium. The minor changes appear to be correlated with the cation-induced increase in variable fluorescence and resolution of the spectra at 77 K reveals that the changes in dichroism are due to LD bands of pigments associated with the light-harvesting complex.

Introduction

The distribution of excitation energy between the two photosystems in the thylakoids of plants and algae can be altered by selective illumination of cells by light absorbed preferentially by either PS I or PS II [1,2] or alternatively following treatment of isolated thylakoids by cations [3]. The same range of concentration of cations also bring about a number of physiological effects some of which are directly related to the redistribution of energy. Such effects include changes in enhancement and the quantum efficiencies of partial electron transfer reactions driven by PS I and PS II [4]. As yet the detailed mechanism for the regulation of this energy redistribution is unknown but both

Abbreviations: PS I, Photosystem I; PS II, Photosystem II; LD, linear dichroism; CD, circular dichroism; EF, fracture face of an exoplasmic membrane leaflet; Chl, chlorophyll.

Bonaventura and Myers [1] and Murata [2] suggested the possibility of the occurrence of conformational changes within the thylakoid that might alter the mutual orientation of pigments and energy transfer between them. Seely [5] has demonstrated theoretically how the reorientation of only a few pigment molecules within a photosynthetic unit could result in such energy redistribution, but an alternative mechanism has been proposed by Barber and coworkers [6] based upon a consideration of electrical diffuse layers and their influence on the membrane.

We have attempted to verify experimentally the hypothesis of Bonaventura and Myers [1] and Murata [2] initially by investigating the effects of cations on the linear dichroism of magnetically aligned thylakoids to search for possible changes in orientation of molecules within the photosynthetic unit. In a preliminary report [7] we noted that low concentrations of bivalent cations caused changes in the LD spectrum of thylakoids that we interpreted as a reorientation of a long wavelength form of Chl *a*. We are aware of the possible contribution of light scattering changes as a result of the bivalent induced thylakoid stacking process [8] leading to distortions as have been found in CD measurements [9—11], but previous workers have implied that polarized scattering had a negligible effect on the LD spectrum of thylakoids [12,13].

We have reinvestigated the effect of cations on the LD of thylakoids with an instrument of higher spectral resolution and also determined the contribution of selective polarized light scattering.

Methods

Envelope-free chloroplasts were isolated from peas as described previously [14] and stored in 300 mM sucrose/1 mM Tris-HCl (pH 8.0). Linear dichroism measurements were made on magneto-oriented specimens at room temperature and at 77° K following trapping of the samples in the oriented condition as described by Vermeglio et al. [15]. The sensitivity of the instrument was much higher than that used previously [7] and the LD signal, $\Delta A = A_{\parallel} - A_{\perp}$, was obtained directly by demodulation of the 100 kHz measuring beam which was alternated between vertically and horizontally polarized light by means of a photoelastic modulator [13,16].

Polarized light scattering, $\Delta S = S_{\parallel} - S_{\perp}$, was measured using the same instrument by monitoring the light scattered directly below the sample in a direction perpendicular to both the polarized measuring beam and the magnetic field but parallel to the thylakoid planes. Fig. 1 depicts the geometric relationship between the thylakoid planes and the viewing directions for acquistition of ΔA and ΔS. No fluorescence artefacts were detected at the chlorophyll concentrations and measuring beam intensities used.

The yield of variable fluorescence, ΔF, was determined from induction experiments [17]. The 680 nm emission was measured following the onset of broad band illumination (values of $\lambda \leqslant 600$ nm) of high intensity. ΔF was obtained by subtraction of the initial yield, F_0, from the maximal yield, F_m, at 5 s.

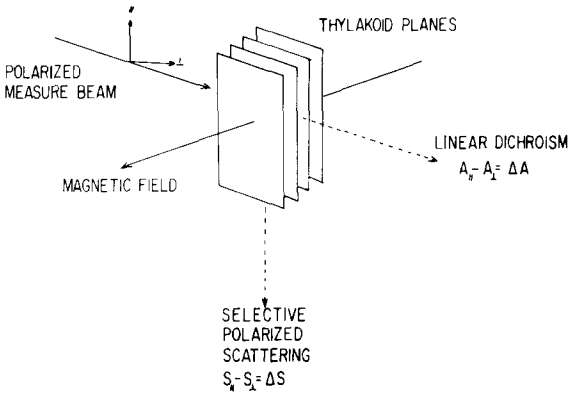

Fig. 1. Direction of the polarized measuring beams in relation to the thylakoid planes, magnetic field (H) and detector positions in the optical measurements.

Results

Fig. 2 shows the effect of a low concentration of Mg^{2+} on the room temperature LD spectrum of thylakoids. As noted previously [7] similar effects were noted upon addition of Ca^{2+} at the same concentration and univalent ions at much higher concentrations (at least 100 mM). The cation-induced modification of the LD spectrum is characterized by an overall change in the direction of greater apparent absorption of vertically polarized light, a red shift and small decreases in signal in the range 650—670 nm, the most prominent one being at 656 nm. The difference spectrum below, $\Delta\Delta A$, shows the cation-induced changes more clearly and the small decrease at 656 nm (ΔA_{656}) is approx. 0.2% of the absorption maximum of the chlorophyll envelope and would not have been detected on our previous instrument [7].

Fig. 3 shows the effect of low concentrations of Mg^{2+} on the polarized scattering of a dilute suspension of oriented thylakoids. The scattering curve is qualitatively similar to the selective scattering spectrum of *Chlorella* reported by Latimer and Rabinowitch [18] and, again, the Mg^{2+}-induced change is attended by an overall change in the direction of an increase in scattering of vertically polarized light (or decrease in S of horizontally polarized light) and a shift of the curve to slightly longer wavelengths. The difference between the two scattering curves ($\Delta\Delta S$) is shown below to indicate the effect of the cation addition.

With the exception of the short wavelength decreases in signal on the blue edge of the thylakoid LD spectrum the difference spectra $\Delta\Delta A$ and $\Delta\Delta S$ shown in Figs. 1 and 2 are qualitatively similar. This indicates that in all probability the large Mg^{2+}-induced change in dichroism can be attributed to the change in scattering resulting from morphological reorganization of the thylakoids during cation-induced stacking [8]. Efforts were made to quantitate the scattering contribution in order to correct the LD spectrum but it was found to be impossible because of the differences in geometry between the sample and detectors in the measurement of ΔA and ΔS and the chlorophyll

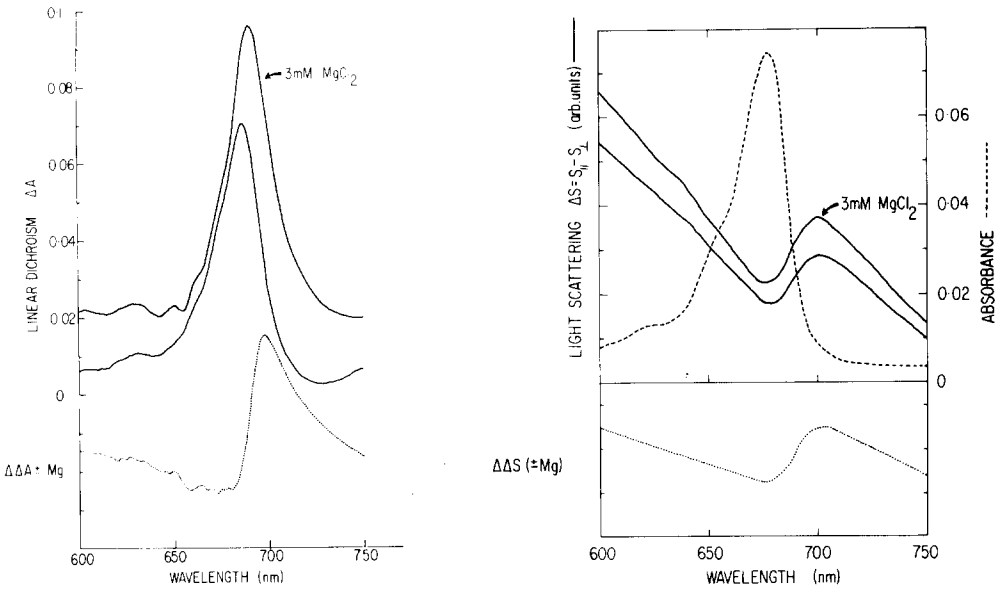

Fig. 2. Room-temperature linear dichroism spectra of pea thylakoids. The thylakoids were suspended in 100 mM sucrose/5 mM Tris-HCl (pH 8.0) and $MgCl_2$ was added as indicated. A magnetic field of 18 kG was used for orientation of the thylakoids which had an absorbance of 1.0 at 675 nm. The difference spectrum below (· · · · · ·) was obtained by subtraction of the two LD spectra and is on the same scale.

Fig. 3. Room temperature absorption spectrum (- - - - - -) and polarized scattering curves (———) of pea thylakoids. Conditions as in Fig. 1 except that the thylakoids were used at a much lower concentration ($A_{675nm}^{10mm} = 0.07$). The difference spectrum below (· · · · · ·) was obtained by subtraction of the two scattering curves.

concentration used in the determinations. Very low concentrations ($A_{675nm}^{10mm} \simeq 0.07$) were used in the measurement of ΔS [18].

Attempts were therefore made to eliminate the light scattering contribution by matching the refractive index of the medium with that of the thylakoids. A variety of agents was used including dimethyl sulfoxide, poly(ethylene glycol) and serum albumin, but the best results were obtained using glycerol. Measurement of the cation-induced changes in ΔA, ΔS and ΔF were then made as a function of glycerol concentration. Both the Mg^{2+}-induced change in ΔA_{656} and increase in ΔF were not significantly affected by glycerol up to 50% v/v (Table I), in contrast to the scattering change which was gradually eliminated by increasing the glycerol concentration. The Mg^{2+}-induced change in ΔA_{656} and ΔF were not observed at glycerol concentrations higher than 50% v/v, suggesting a correlation and possible physiological relevance of the small change in dichroism that is partially separable from the scattering change.

As the regulation of energy distribution as measured by cation-induced increase in ΔF was not seriously impaired by addition of 50% glycerol to the medium it was decided to perform the LD measurements at the temperature of liquid N_2 to increase the resolution of the spectra. The glycerol addition effectively eliminated the formation of ice crystals during cooling which would otherwise have depolarized the measuring beam [15]. Fig. 4 shows LD spectra

TABLE I

EFFECT OF GLYCEROL CONCENTRATION ON THE Mg^{2+} INDUCED INCREASES IN SCATTERING AND VARIABLE YIELD FLUORESCENCE AND DECREASE IN LD AT 656 nm

Glycerol (% v/v)	ΔS (% control)	ΔF (arbitrary units)	ΔA_{656} ($\times 10^3$)
0	100	18	4.6
20	98	18	4.6
30	79	17	4.5
40	62	17	4.2
50	44	14.4	4.0
60	18	1.7	0
70	0	0	0

of thylakoids at 77 K following magneto-orientation at room temperature and then slow cooling to trap the samples in the oriented condition. The Mg^{2+}-induced changes are most clearly seen in the difference spectrum below ($\Delta\Delta A$). The positive peak of the scattering contribution is centered at 690 nm and is much reduced due to the glycerol addition but the small short-wavelength changes are relatively enhanced and resolved at 650, 658 and 670 nm. We have recently reported on the orientation of Chl *a* and Chl *b* forms on isolated

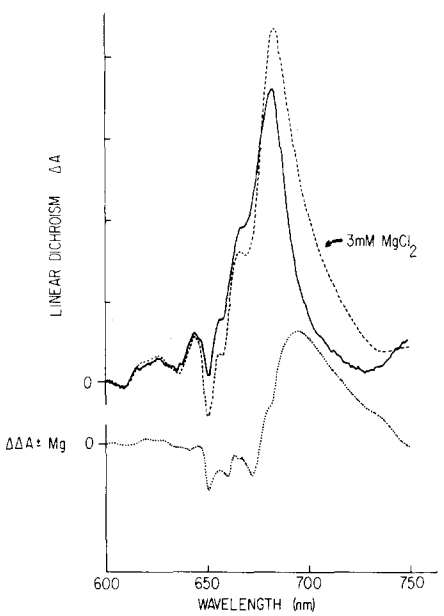

Fig. 4. 77 K linear dichroism spectra of pea thylakoids. The suspension medium of the thylakoids (A^{1mm}_{675nm} = 0.2, room temperature) was supplemented with glycerol (50% v/v). The samples were oriented in a magnetic field of 18 kG and then trapped by freezing slowly in liquid nitrogen. The spectra with and without Mg^{2+} are of different but representative samples of the two treatments and the difference spectrum below (· · · · · ·) was obtained by subtraction of the two LD spectra shown and is on the same scale. Each division on the ΔA ordinate is 0.03 and the enhancement in total signal over that at room temperature is approximately 6.

pigment-protein complexes from thylakoids aligned in stretched poly(vinyl alcohol) films [16]. From such data it is possible to assign the Mg^{2+}-induced changes here to those resulting from dichroism of the light-harvesting complex. The negative LD bands at 77 K at 650 and 658 nm are due to Chl *b* and a Chl *a* form, respectively, of which the Q_y transition moments are oriented in a plane perpendicular to the long geometric axis of the light-harvesting complex and the 670 nm band is due to a Chl *a* form, the orientation of which is parallel. Although the situation is complex, it is conceivable that the small Mg^{2+}-induced changes in ΔA at short wavelengths might be indicative of a change in orientation of the light-harvesting complex itself or a few molecules within the light-harvesting complex.

Discussion

The data presented above confirm our previous report [7] that the addition of bivalent cations in concentrations known to affect the distribution of excitation energy between the two photosystems, causes changes in the LD of thylakoids. Comparison of the difference spectra $\Delta\Delta A$ and $\Delta\Delta S$ in Figs. 2 and 3 show that, with the exception of some small decreases in signal on the short wavelength side of main LD band, the cation-induced modification of the LD spectrum can be attributed to polarized light scattering. The small changes that are resolved in the low-temperature spectrum in Fig. 4 are in all probability due to a change in dichroism of the light-harvesting complex which represents a large fraction of the chlorophyll *a* and most of the chlorophyll *b* [19] and which is visualized by electron microscopy as particles in association with the PS II core on the EF face [20]. The cation-induced change in LD of the light-harvesting complex appears to be distinct from the scattering change resulting from thylakoid stacking in that the scattering contribution is gradually reduced by increasing the refractive index of the medium whereas the LD change is not affected until there is an inhibition of the fluorescence of variable yield.

Previous investigators have noted the existence of selective polarized scattering by thylakoids and algae [12,22,23] but have reported that the effects do not seriously distort the LD spectrum [12,13]. As expeceted, Breton et al. [12] showed that the addition of glycerol removes the distortion at long wavelengths but concluded that the scattering effects did not prevail in their measurements. This was affirmed in subsequent work by Gagliano et al. [13] who showed similarities in the electric dichroism of whole thylakoids and subchloroplast fragments despite the great difference in particle size. Such conclusions were also supported by the observations of Faludi-Dániel and Breton [24] on the LD of granal and agranal chloroplasts. Although they found small differences most probably due to different amounts of Chl a_{670} and Chl a_{691}, the degree of thylakoid stacking was not reflected in the LD, which appears to be in contrast to CD measurements [9—11].

Swenberg and Geacintov [25] analyzed the selective polarized light scattering by oriented photosynthetic membranes and presented theoretical scattering curves that agree very well with those measured from *Chlorella* by van Nostrand [23]. It is not possible to compare the scattering data here with those of these

investigators because of the difference in data acquisition. Swenberg and Geacintov [25] reported on the wavelength dependence of the differential scattering ratios

$$S_1 = S_{\parallel}(\lambda,H)/S_{\parallel}(\lambda,0)$$

and

$$S_2 = S_{\perp}(\lambda,H)/S_{\perp}(\lambda,0)$$

S_1 and S_2 are defined in terms of orientation of the membranes and the directions of polarization and viewing of the scattered light, and $S_{\parallel}(\lambda,H)$ and $S_{\perp}(\lambda,H)$ refer to scattered light polarized parallel and perpendicular, respectively, to the membrane planes. In the measurements reported here and as shown in Fig. 1 the scattering of both vertically and horizontally polarized incident light was viewed parallel to the thylakoid planes, which provides the relevant term in an assessment of the contribution of scattering to the ΔA signal. A more detailed study of ΔS by thylakoids is desirable but beyond the scope of this contribution.

Although it was not possible to eliminate completely the cation-induced scattering change without seriously impairing the regulation of energy distribution in the thylakoids, it does appear that the minor short wavelength changes in LD truly reflect changes in dichroism of the light-harvesting complex. The physiological relevance of the cation-induced change in LD of the light-harvesting complex remains to be determined but it has been established by analysis of the state 1—2 transition in vivo [26] and the cation-induced changes in isolated thylakoids [21] that the light-harvesting complex is required in the control of energy distribution. The data here are consistent with either a small proximity change of the light-harvesting complex with respect to the thylakoid plane or a conformational change of some pigments within the light-harvesting complex itself. The effects noted here are very rapid compared with the long-term particle migration and re-arrangement on the EF face but are within the time-scale of the initial thylakoid adhesion process [27]. The data cannot discriminate between whether the minor LD changes observed are a result of the stacking phenomenon or because of direct implication in the control of the energy distribution process. With respect to the latter, the hypothesis of Bonaventura and Myers [1] and Murata [2] is not ruled out and our present studies on pigment orientation of units undergoing energy redistribution in the absence of gross changes in thylakoid architecture such as during the state 1—2 transition [28] should have a bearing on the validity of this supposition.

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