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# REQUIREMENT OF THE LIGHT-HARVESTING PIGMENT · PROTEIN COMPLEX FOR MAGNESIUM ION REGULATION OF EXCITATION ENERGY DISTRIBUTION IN CHLOROPLASTS

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## Summary

Cation regulation of excitation energy distribution was examined in chloroplasts isolated from (a) pea seedlings, grown in intermittent illumination, which contain no light-harvesting complex, (b) a barley mutant which is deficient in the major polypeptide component of the light-harvesting complex, and (c) a soybean mutant which contains a reduced amount of light-harvesting complex. It was found that:

(1) $Mg^{2+}$-induced increase in Photosystem II fluorescence at room temperature is small in the chloroplasts of the soybean mutant, smaller in the barley mutant, and almost absent in the light-harvesting complex-less chloroplasts of pea as compared to their respective controls.

(2) $Mg^{2+}$-induced increase in the $F_{685}/F_{730}$ emission peak ratio at 77 K is not detected in the isolated chloroplasts of the intermittent light-grown pea and the barley mutant.

(3) Pre-illumination induced State 1-State 2 adaptation in vivo is absent in the barley mutant and is less pronounced in the soybean mutant as compared to their respective controls.

(4) Increase of slow fluorescence decay upon addition of $Mg^{2+}$ observed in control chloroplasts was not detected in chloroplasts of intermittent-light grown peas.

These results confirm earlier conclusions (Armond, P.A., Arntzen, C.J., Briantais, J.-M. and Vernotte, C. (1976) Arch. Biochem. Biophys. 175, 54—63; Davis, D.J., Armond, P.A., Gross, E.L. and Arntzen, C.J. (1976) Arch.

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Abbreviations: SDS, sodium dodecyl sulfate; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; PS I, Photosystem I; PS II, Photosystem II; Chl, chlorophyll.

Biochem. Biophys. 175, 64—70) that light-harvesting complex is required for the $Mg^{2+}$-induced regulation of the excitation energy distribution between Photosystems I and II.

The characteristic P-S decay and I-D dip of the in vivo fluorescence inductions (Kautsky effect) were not significantly altered in the light-harvesting complex-less and the light-harvesting complex-deficient chloroplasts as compared to their respective controls. These results indicate that light-harvesting complex is not obligatorily required to observe the P-S decay or the I-D dip.

## Introduction

Chlorophylls and other light-harvesting pigments of chloroplast lamellae are functionally organized to give a high degree of efficiency in transfer of absorbed excitation energy from the pigment bed to the reaction centers. Since most chlorophylls are associated with proteins within the membrane [1], the structural organization of pigment · protein complexes must play a major role in determining photosynthetic quantum efficiency. To explain the kinetic properties of fluorescence which are associated with the excitation energy distribution between Photosystems I and II, Butler and co-workers [2,3] proposed a tripartite model for the photosynthetic apparatus which includes separate pigment · protein complexes for Photosystems I and II in addition to a light-harvesting chlorophyll · protein complex. The latter was suggested to act as a functional component which can transfer excitation energy to either Photosystem and can participate in energy transfer from Photosystem II to Photosystem I.

During the last decade much evidence has accumulated which indicates that $Mg^{2+}$-induced regulation of the excitation energy distribution between the Photosystems is important in regulating the quantum efficiency of photosynthesis [4—9]. It has recently been suggested [10,11,17] that a photochemically inactive light-harvesting complex, containing the chlorophyll *a/b* protein of Thornber [1], is involved in the $Mg^{2+}$-induced regulation of energy transfer. During the greening of pea chloroplasts, the appearance of the light-harvesting complex in the membranes was demonstrated to parallel the appearance of the $Mg^{2+}$-induced regulation of energy transfer. In membrane fractionation studies, the light-harvesting complex was found to bind reversibly to the Photosystem II complex via cation-mediated interactions; the $Mg^{2+}$ concentration required for such binding was directly related to the $Mg^{2+}$ concentration required for the regulation of energy distribution.

In this paper we have studied in more detail the fluorescence characteristics related to energy distribution of isolated chloroplasts and intact leaves in three systems, namely, the intermittent-light grown pea and a mutant barley (both lacking the pigmented light-harvesting complex), and a mutant soybean containing a reduced amount of the light-harvesting complex. We will provide new evidence for the role of light-harvesting complex in cation-mediated regulation of energy distribution. It will also be demonstrated that the State 1-State 2 transition in vivo is also dependent on the presence of the light-harvesting complex.

## Materials and Methods

Pea (*Pisum sativum* cv. Progress No. 9) and soybean (*Glycine max* cv. Clark) seedlings were grown under cool-white fluorescent light, 16-h photoperiod, in vermiculite moistened with half-strength Hoagland's solution. (Soybean seedlings of the $Y_{11}y_{11}$ genotype were grown under daylight Power Groove fluorescent light (General Electric Co.)). Barley (*Hordeum vulgare*) seeds kindly supplied by Dr. Jan M. Anderson were grown in soil moistened alternately with deionized water and half-strength Hoagland's solution.

Chloroplasts were isolated as previously described [12] except that the sucrose washes were omitted. In the case of soybean and barley, the grinding medium also contained 0.25% bovine serum albumin (fraction V) and 0.05 M sodium ascorbate; centrifugation was carried out at 10 000 × *g* for 2 min and the resuspension medium also contained 1% defatted bovine serum albumin, fraction V, 0.05 M sodium ascorbate, and 0.1 M sorbitol. "Intermittent light plastids" were prepared as previously described [12].

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of membrane samples was carried out as described by Arntzen and Ditto [12], with a current of 6 mA/gel for 30 min.

Measurements of fluorescence at 90° angle to incident light were made with an Aminco J10-280 photomultiplier microphotometer with an S-20 response phototube. A Corning CS 2-64 (sharp cut-off red) filter was mounted in front of the phototube. The sample was illuminated by a Vickers Intense Lamp with a regulated, low-ripple output power supply. The light was filtered by Corning CS 1-75 (heat absorbing) and CS 4-96 (broad-band blue) filters. Exposure of samples was controlled by a Copal photographic shutter which gave an opening time of less than 1 ms. Measurement of $F_0$, the immediate fluorescence level, was taken within 2 ms after full shutter opening and $F_M$, the maximum total fluorescence, was measured after addition of 10 $\mu$M DCMU to chloroplast samples.

Fluorescence emission spectra at 77 K were measured in an Aminco-Bowman spectrofluorimeter at an exciting wavelength of 440 nm (30 nm half-band width). The observation (emission) wavelengths were scanned with an exit slit adjusted to give a 3 nm half-band width.

Chlorophyll *a*/*b* ratios were determined after methanol extraction using the equations given by Holden [13].

## Results

*Light-harvesting complex content in the systems studied*

The concentration of pigment · protein complexes within chloroplast membrane was measured by polyacrylamide gel electrophoresis in pea, barley and soybean (Table I). Control chloroplast lamellae solubilized in SDS and subjected to polyacrylamide gel electrophoresis yielded three pigmented bands, identified as Complex I, the light-harvesting complex, and a solubilized, free pigment band [1,12]. In contrast, the intermittent-light grown peas [11] and a mutant barley [14] which are deficient in chlorophyll *b* contained no light-harvesting complex. As had previously been reported [15], the soybean mutant

TABLE I

COMPARISON OF PIGMENT · PROTEIN COMPLEXES IN SDS-SOLUBILIZED MEMBRANES

For details, see Materials and Methods.

| Sample | Chl *a*/Chl *b* | % of total chlorophyll in band | | |
|---|---|---|---|---|
| | | Complex I | Light-harvesting complex | Free pigment |
| Pea | | | | |
| Control | 2.8 | 20 | 46 | 35 |
| Intermittent-light grown | >30 | 19 | 0 | 81 |
| Barley | | | | |
| Wild type | 3.1 | 15 | 27 | 58 |
| Mutant | >30 | 13 | 0 | 87 |
| Soybean | | | | |
| Wild type | 3.8 | 21 | 32 | 47 |
| $Y_{11}y_{11}$ | 7.6 | 20 | 18 | 63 |

($Y_{11}y_{11}$) is deficient in chlorophyll *b*; it also is shown to be partially deficient in light-harvesting complex. It will be demonstrated in the following sections that the degree of $Mg^{2+}$-induced regulation of excitation energy distribution depends on the relative amount of light-harvesting complex present in the membrane.

*Fluorescence yield at room temperature*

Fig. 1 illustrates the effects of $Mg^{2+}$ on the fluorescence yield of chlorophyll *a* in isolated chloroplasts of control and intermittent-light grown peas.

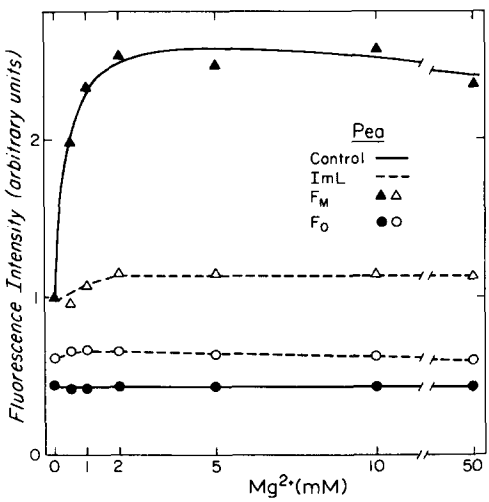



Fig. 1. Effect of $MgCl_2$ on chlorophyll *a* fluorescence of pea chloroplasts. Separate suspensions were prepared for each $Mg^{2+}$ concentration at a constant chlorophyll concentration (5—15 μg/ml in different experiments) in a reaction medium consisting of 0.1 M sorbitol/0.5 mM methyl viologen/10 mM NaCl/1 mM sodium tricine, pH 7.8. Suspensions, with $Mg^{2+}$, were prepared 15 min prior to fluorescence measurement and held in an ice-bath. The $F_M$ values for the control and intermittent-light grown (ImL) chloroplasts were normalized to unity at 0 mM $Mg^{2+}$.

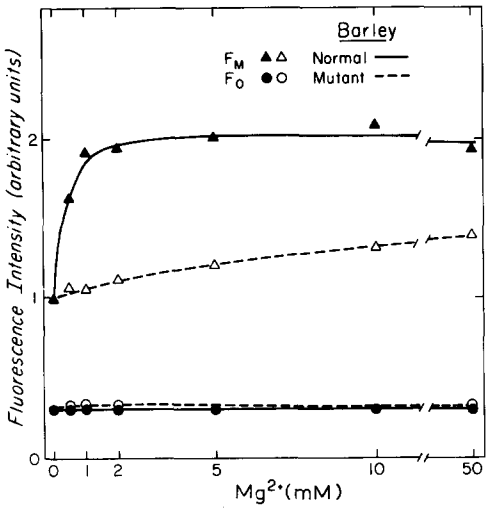



**Fig. 2. Effect of $MgCl_2$ on chlorophyll *a* fluorescence of barley chloroplasts. Samples were prepared as indicated in the legend of Fig. 1.**

Fluorescence at room temperature has two components, a very fast rising fluorescence ($F_0$), probably arising from the light-harvesting chlorophylls, and a variable fluorescence component ($\Delta F$) which arises from Photosystem II and is dependent on the redox state of Q [16]. In the presence of DCMU, which blocks electron flow from Photosystem II to the intersystem electron carriers, any change in steady state fluorescence ($F_M$) of a given chloroplast sample is generally regarded as a consequence of change in the input of excitation energy into the Photosystem II reaction centers [16]. In agreement with earlier studies [5—9], $Mg^{2+}$ elicited a large increase in the variable fluorescence in control pea (Fig. 1), barley (Fig. 2), or soybean chloroplasts. This has been interpreted as indicating that a greater fraction of absorbed energy is distributed into the Photosystem II centers in the presence of $Mg^{2+}$ than in its absence. With intermittent-light grown chloroplasts, $Mg^{2+}$ had very little effect on either $F_0$ or $F_M$, thus indicating that $Mg^{2+}$ exerts almost no regulation of excitation energy distribution in the absence of the light-harvesting complex. Similar relative effects of $Mg^{2+}$ were obtained with barley chloroplasts (Fig. 2). At 2—3 mM $Mg^{2+}$ the fluorescence enhancement was saturated in normal barley chloroplasts. At this concentration of $Mg^{2+}$ the fluorescence enhancement with the mutant barley chloroplasts was only about 10% of the enhancement observed with normal chloroplasts. In mutant chloroplasts, however, a further increase of fluorescence was observed at higher concentrations of $Mg^{2+}$. In mutant soybean chloroplasts, a qualitatively similar but smaller $Mg^{2+}$ effect was observed as compared to the control soybean plastids. (In Figs. 1 and 2, the $F_M$ values in the absence of $Mg^{2+}$ are normalized; therefore, comparison of $F_0$ values between normal and light-harvesting complex-less chloroplasts would be misleading).

*Fluorescence at 77 K in isolated chloroplasts*

At 77 K the fluorescence emission peak at approx. 730 nm, originating

TABLE II

EFFECT OF $Mg^{2+}$ ON 77 K FLUORESCENCE OF CHLOROPLASTS

Samples were prepared as indicated in the legend of Fig. 1.

| Sample | $Mg^{2+}$ (mM, at fluorescence ratio $F_{685}/F_{730}$ | | | |
|---|---|---|---|---|
| | 0 | 2 | 5 | 50 |
| Pea | | | | |
| Control | 0.6 | 1.1 | 1.2 | 1.2 |
| Intermittent-light grown | 2.8 | 2.8 | 2.6 | 2.7 |
| Barley | | | | |
| Wild type | 1.4 | 2.5 | 2.9 | 2.9 |
| Mutant | 1.4 | 1.5 | 1.6 | 1.6 |

from Photosystem I (PS I), is pronounced; since electron transport is blocked at low temperatures, the ratio of fluorescence emitted at 685 nm (arising mainly from Photosystem II (PS II)) to that emitted at 730 nm provides an indication of the change of distribution of excitation energy between the Photosystems [5,16]. It has been shown previously [5,7,10,16,17] from the relative fluorescence intensities at 685 and 730 nm in the presence and absence of $Mg^{2+}$ that an increase in energy input into the PS II resulted from a decrease of PS I energy input. Table II shows that with control chloroplasts of pea and barley, the $F_{685}/F_{730}$ ratio increased upon addition of $Mg^{2+}$, indicating a relative increase of energy input into PS II. With intermittent-light grown and mutant barley chloroplasts no significant change in the ratio was observed upon $Mg^{2+}$ addition.

It is to be noted that the magnitude of the $F_{685}/F_{730}$ ratio in intermittent-light grown chloroplasts was significantly higher than the control value either in the presence or absence of $Mg^{2+}$. This increased ratio could be attributed to one or several of the following: (a) a decrease of the fluorescence yield of PS I, (b) a decrease or absence of spillover from PS II to PS I, (c) absence of energy transfer between PS II units, and (d) less reabsorption of 685 nm fluorescence occurring in intermittent-light grown chloroplasts. The second possibility might be eliminated on the basis of previous observations that spillover can occur in the absence of light-harvesting complex [17,18]. The first possibility is supported directly from the observation that the fluorescence yield of PS I is higher in the presence of light-harvesting complex (Butler, personal communication). The absence of sigmoidal rise of fluorescence in the intermittent-light grown chloroplasts [10] supports the third possibility. If the light-harvesting complex is assumed to contribute to the high degree of packing of pigments in the PS II, it is conceivable that 685 nm fluorescence would be more extensively reabsorbed in the control than in the intermittent-light grown peas.

*State 1-State 2 adaptation*

The State 1-State 2 phenomenon has been interpreted as being due to a time-dependent redistribution of excitation energy between the Photosystems. Myers and co-workers [20—22] demonstrated that a preillumination of

TABLE III

STATE 1/STATE 2 PHENOMENON (EFFECT OF PRE-IRRADIATION ON 77 K FLUORESCENCE OF CHLOROPLASTS)

Preilluminations were carried out as previously described [23] using a 650 ± 6 or 710 ± 7 nm, 3-cavity interference filter in combination with a Corning 1-75 (heat absorbing) filter at intensities of 5.0 $\mu$W $cm^{-2}$ and 6.8 $\mu$W $cm^{-2}$, respectively. Leaf samples were preilluminated on both surfaces simultaneously for 15 min, then immediately ground in a chilled Waring blender in a solution of 0.4 M sorbitol/0.1 M sodium tricine, pH 7.8/0.1% glutaraldehyde. The resulting brei was filtered through 4, then 12 layers of cheese-cloth and centrifuged for 10 min at 3000 × $g$, and the pellet resuspended in 60% glycerol.

| Sample | $F_{685}/F_{730}$ after pre-irradiation at | | % Change $(\frac{a-b}{a} \times 100)$ |
|---|---|---|---|
| | 710 nm (a) | 650 nm (b) | |
| Barley | | | |
| Wild type | 2.5 | 1.6 | 36 |
| Mutant | 1.3 | 1.4 | — |
| Soybean | | | |
| Wild type | 2.1 | 1.7 | 19 |
| $Y_{11}y_{11}$ | 2.8 | 2.6 | 7 |

*Chlorella* cells with PS I light (near 710 nm) resulted in increased sensitization of PS II (State1), whereas preillumination with PS II light (near 650 nm) elicited opposite effects (State 2). The State 1-State 2 phenomenon has also been demonstrated in rapidly isolated chloroplasts from preilluminated pea leaves [23]. In the latter experiments, plastids were fixed with glutaraldehyde during tissue disruption and the samples were then examined for fluorescence emission spectra at 77 K. Using similar techniques we calculated the ratio of 685 nm emission to 730 nm emission of chloroplast samples following preillumination with 710 or 650 nm light. Table III shows a higher $F_{685}/F_{730}$ ratio in State 1 (preillumination by 710 nm) than in State 2 (preillumination by 650 nm) in the case of normal chloroplasts of barley and soybean. In contrast, the state change phenomenon was not detected in the barley mutant and was observed with decreased magnitude in the soybean mutant $Y_{11}y_{11}$. These results suggest that light-harvesting complex is involved in in vivo energy redistribution during State 1-State 2 adaptation.

It has been suggested that the State 1 in whole cells corresponds to the state with $Mg^{2+}$ in isolated chloroplasts and the State 2 to the state without $Mg^{2+}$ in terms of the relative intensities of $\Delta F$, sigmoidicity of the induced fluorescence rise, and action spectra of whole-chain electron flow [23,24]. Thus, $Mg^{2+}$ flux is thought to be involved in the State 1-State 2 phenomenon. From this point of view our observation that the State 1-State 2 phenomenon is absent in a system lacking the light-harvesting complex is in agreement with the idea that $Mg^{2+}$-induced regulation of excitation energy distribution is dependent upon light-harvesting complex.

*Slow fluorescence decay*

Sokolove and Marsho [39] have shown that slow fluorescence quenching in isolated chloroplasts involves two, independent, component mechanisms. One of these, previously observed in osmotically shocked chloroplasts by Krause

TABLE IV

SLOW FLUORESCENCE DECAY IN PEA CHLOROPLASTS

100% measuring light intensity was $3.4 \cdot 10^4$ erg $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$. For details see Fig. 1.

| Sample | Measuring light intensity | % Quenching of fluorescence during 3 min of illumination | |
|---|---|---|---|
| | | No $MgCl_2$ | 5 mM $MgCl_2$ |
| Pea, control | 100% | 4 | 25 |
| | 11% | 0 | 14 |
| Pea, intermittent-light grown | 100% | 23 | 23 |
| | 15% | 0 | 0 |

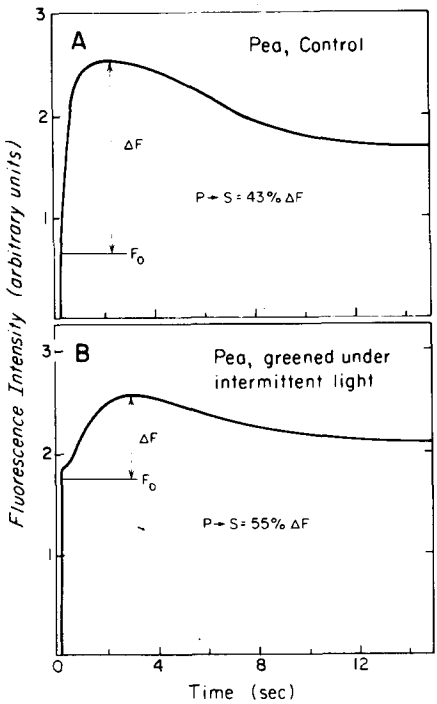
[37] and Barber and Telfer [40], was largely dependent on the presence of low levels of divalent cations (approx. 5 mM $Mg^{2+}$) or higher levels of monovalent cations (approx. 150 mM $Na^+$ or $K^+$), and was sensitive to the uncouplers of photophosphorylation and the cation-specific ionophores, especially that for $Mg^{2+}$. It has been suggested that this mechanism consists of cation efflux during illumination, resulting in a redistribution of excitation energy.

The second mechanism, which was observed by Jennings et al. [38], was insensitive to uncouplers and ionophores but intensity-dependent, being virtually absent at sufficiently low intensities of illumination but becoming predominant at higher intensities. The maximum intensity used by Jennings et al. was 100 kerg $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$, but the intensity requirement of this mechanism may be highly dependent upon the concentration and light-harvesting characteristics of the chloroplasts, and the wavelengths of exciting light used.

We examined the effect of $Mg^{2+}$ on slow fluorescence quenching in control and intermittent-light grown chloroplasts of pea (Table IV). In the absence of $Mg^{2+}$, control chloroplasts showed little or no slow fluorescence quenching at either of the light intensities used, but significant quenching was observed in the presence of $Mg^{2+}$, even at the lower intensity. Intermittent-light grown chloroplasts showed no effect of $Mg^{2+}$ on slow fluorescence quenching at either 100% (34 kerg $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$) or 15% intensity. The $Mg^{2+}$-insensitive quenching observed at 100% intensity in these chloroplasts can be ascribed to the high-light mechanism observed by Jennings et al. [38]. Since formation of proton gradients has been observed in intermittent-light grown chloroplasts [10], we interpret the absence of slow fluorescence quenching in 15% light as being due to absence of $Mg^{2+}$-induced redistribution of excitation energy upon illumination, resulting from the absence of light-harvesting complex. The two types of chloroplasts cannot be compared as to the amounts of quenching observed because the light intensity used may not be the same in relation to the saturation intensity for each system. In any case, the $Mg^{2+}$ insensitivity of slow fluorescence quenching in the intermittent-light grown chloroplasts suggests that light-harvesting complex is required for $Mg^{2+}$-induced changes in slow fluorescence quenching.

*Fluorescence transients in whole leaves; the P-S decay*

Upon illumination of dark-adapted, intact, photosynthetic leaf tissue, a



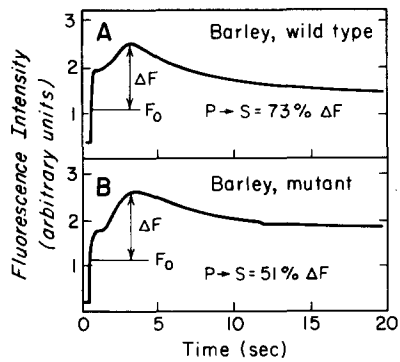




**Fig. 3. Fluorescence transients of excised pea leaves. For details see Materials and Methods.**

**Fig. 4. Fluorescence transients of excised barley leaves. For details see Materials and Methods.**

series of transient changes in fluorescence occurs (the Kautsky effect) [25]. Following an immediate fluorescence rise to the "O" level, there is a rapid increase to an intermediate (I) fluorescence intensity. This can be followed by a decline (to a dip D) and a slower increase to a peak (P) or maximal fluorescence intensity. Subsequently, the fluorescence decreases to a semi-steady state level (S).

The P-S decay of the fluorescence transient in photosynthetic cells is thought to be linked to the high energy state and cation redistribution in thylakoid membranes [25,26]. Therefore, it was of interest to study the involvement of light-harvesting complex in P-S decay in higher plant tissues varying in light-harvesting complex content. Contrary to expectations, the P-S decay was observed with light-harvesting complex-less or -deficient leaves of pea and barley (Figs. 3 and 4). The P-S decay as measured by percent quenching of variable fluorescence in the light-harvesting complex-deficient pea leaves was of similar magnitude as in the control. The quenching of variable yield fluorescence in the mutant barley was two-thirds that of its control. These observations suggest that the P-S decay in vivo is not identical to the State 1-State 2 transition in vivo or in isolated chloroplasts.

*I-D decline of fluorescence transient in leaves*

The characteristic I-D decline of a fluorescence transient curve (Kautsky effect) has been studied in whole cells [27,28], intact chloroplasts [29] and

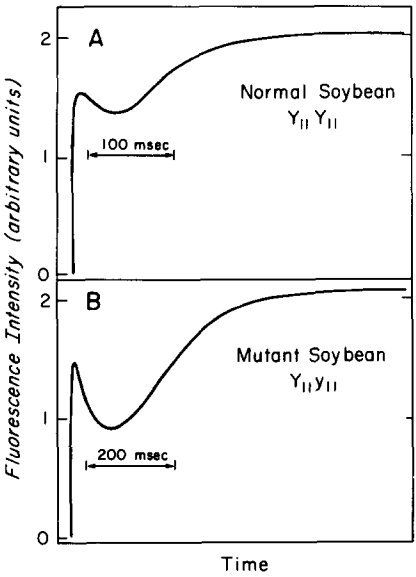



Fig. 5. Fluorescence transients of excised soybean leaves. Leaves were irradiated with strong, blue light for 1 s, dark-adapted for 3 s and then illuminated with blue light again as the fluorescence transients were recorded. This treatment accentuated the I-D transition.

leaves [30]. It is generally agreed that the I-D decline represents, at least partially, the reoxidation of Q linked to PS I activity. Schreiber and Vidaver [30] measured directly the ratio of fluorescence emitted at $\lambda < 690$ to $\lambda > 710$ nm and demonstrated a transient in the fluorescence ratio in 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-poisoned systems. This transient occurred on the same time scale as the I-D dip. They interpreted the I-D decline to be partly caused by a rapid redistribution of excitation energy to PS I. Their interpretation was based on the tripartite model [2] which incorporates light-harvesting complex that can transfer excitation energy to either of the Photosystems.

We studied the fluorescence transient in pea, barley and soybean leaves after a short (1 s) preillumination. (Preillumination has previously been demonstrated to accentuate the I-D dip [27,29]; this was confirmed in our preliminary studies.) Fig. 5 shows a prominent I-D decline in the mutant as well as in the normal soybean. The magnitude of the dip was even greater in mutant than in normal soybean chloroplasts even though the former are partially light-harvesting complex-deficient. A pronounced I-D dip was also observed in intermittent-light grown pea leaves and mutant barley leaves treated as described in Fig. 5. These results indicate that light-harvesting complex is not required for the I-D transient, and this transient is not a result of cation regulation of energy transfer. It is to be noted that the I-D dip was not pronounced at the first illumination of the dark adapted leaves. A pronounced I-D dip required preillumination in all samples. This is consistent with similar observations in intact chloroplasts [29].

## Discussion

It has been shown that a photochemically inactive pigment · protein complex can be isolated from normal, mature chloroplast lamellae [12]. This light-harvesting complex has a chlorophyll *a*/*b* ratio of 1.3 and contains the pigment · protein referred to by Thornber [1] as the light-harvesting chlorophyll *a*/*b* protein. The complex is presumably an aggregate of several individual polypeptides and chlorophylls since it has an estimated diameter of 50—100 Å [12]. The aggregate could not be isolated from chloroplasts of pea seedlings grown under intermittent light.

The light-harvesting complex is thought to contain two major polypeptides in the 23—30 kdalton size range ([31,32] reviewed by Boardman et al. [33]). Both of these polypeptides are absent in intermittent-light grown chloroplasts, whereas the chlorophyll *b*-deficient barley mutant chloroplasts are deficient in only one of the two polypeptides [33,34]. The $Y_{11}y_{11}$ soybean mutant chloroplasts are partially deficient in both polypeptides (unpublished data).

In this study, we have analyzed the pattern of excitation energy distribution in photosynthetic lamellae containing various amounts of light-harvesting complex or its components using fluorescence techniques. Three lines of evidence suggest that the light-harvesting complex, or some components of it, are required for cation regulation of excitation energy distribution. First, the magnitude of $Mg^{2+}$ stimulation of chlorophyll fluorescence at room temperature was a function of light-harvesting complex content (Figs. 1 and 2). Intermittent-light grown pea chloroplasts, which have no pigmented light-harvesting complex and which are missing both major polypeptides associated with the complex [11,35], show a negligible fluorescence increase upon $Mg^{2+}$ addition. The chlorophyll *b*-less mutant of barley, which contains no pigmented light-harvesting complex (Table I and ref. 1), but which does contain one of the two major polypeptides of the light-harvesting complex [33], showed a small fluorescence increase in response to $Mg^{2+}$. It should be noted, however, that this $Mg^{2+}$ response did not saturate over the concentration range tested (up to 50 mM), whereas the response saturated at 2 mM $Mg^{2+}$ in wild-type barley chloroplasts. Mutant soybean chloroplasts, which are partially deficient in light-harvesting complex content, showed a partial increase in fluorescence in response to $Mg^{2+}$; the effective concentration range for $Mg^{2+}$ effects was similar in normal and mutant chloroplasts.

A second approach to quantifying the cation control of energy distribution was to analyze 77 K fluorescence spectra. Shifts in fluorescence emission peak heights, due to changes in energy distribution to Photosystems I and II, were observed in response to $Mg^{2+}$ addition in control, but not in intermittent-light grown pea chloroplasts. Small changes were observed in the barley mutant chloroplasts.

The third measure of regulation of energy distribution was analysis of State 1 and State 2 adaptations of chloroplasts in vivo. A substantial change in the 77 K fluorescence emission spectrum occurred in chloroplasts from preilluminated leaves of wild-type soybean and barley seedlings in response to 650 vs. 710 nm irradiation. The soybean mutant, with partial light-harvesting complex content, showed a reduced State 1-State 2 change, whereas no change

could be detected in the barley mutant. It should be noted that these data support the concept that State 1-State 2 phenomena are directly related to cation control of energy distribution, as has earlier been suggested [23,24].

We conclude that a complete light-harvesting complex is required for full cation regulation of excitation energy distribution between Photosystems I and II. The barley mutant, containing a partial light-harvesting complex polypeptide complement, exhibits only a small $Mg^{2+}$ effect on energy distribution. This is in agreement with previous data of Boardman and Thorne [36]. It is also evident that the magnitude of the cation-induced effects on energy distribution is related to total light-harvesting complex content in the membrane. The $Y_{11}y_{11}$ soybean chloroplasts, which contain reduced amounts of otherwise normal light-harvesting complex, have a reduced cation response as compared to wild type soybean chloroplasts.

We also examined in vivo fluorescence transients of the leaf tissues containing chloroplasts with different levels of light-harvesting complex. Quantitatively, there were small differences in the various sample pairs. In particular, the fluorescence rise times (to a maximal level of fluorescence) were often shorter in samples containing light-harvesting complex. This probably relates to the larger photosynthetic unit size of these plastids. Qualitatively, however, all fluorescence transients were similar.

The mechanism(s) of P-S decay and I-D dip is poorly understood. It has been suggested that P-S decay occurs, to a large extent, as a result of energy redistribution between PS I and PS II (see refs. 22 and 25 for review and alternative interpretations). Energy redistribution between the Photosystems has been suggested also to be involved in the I-D dip [30]. We have shown in this paper that $Mg^{2+}$ regulation of slow fluorescence decay in isolated chloroplasts was obligatorily linked to the presence of light-harvesting complex, while the P-S decay in vivo was independent of light-harvesting complex. These results indicate that the mechanisms of the two phenomena are not identical. While both phenomena could still be related to cation movements, they differ in the requirement of light-harvesting complex.

Unfortunately, these data do not allow an interpretation of what the P-S decay and I-D dip mean in terms of photochemical or membrane regulatory processes. The mutant or intermittent-light grown plants may provide a useful system in which to pursue this problem, since involvement of light-harvesting complex in excitation energy distribution regulation by cations can be removed from consideration.

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