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EVIDENCE FOR ENERGY MIGRATION FROM PHOTOSYSTEM I TO PHOTOSYSTEM II AND THE EFFECT OF MAGNESIUM

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SUMMARY

1. With *Euglena* chloroplasts 722 nm light excites a fluorescence induction pattern, measured at 681 nm, typical of the Photosystem II reduction of Q.

2. Both methylviologen and phenazine methosulfate partially quench this fluorescence rise in the absence of electron transport between Photosystem II and Photosystem I, and in a manner consistent with their known role of stimulating Photosystem I photochemistry.

3. The variable fluorescence excited with 722 nm light is reduced to a greater extent than that excited by 638 nm light upon chlorophyll dilution during division in the dark.

4. These observations are interpreted to indicate that in *Euglena* chloroplasts light absorbed by Photosystem I is transferred energetically "uphill" to Photosystem II, where it can perform Photosystem II photochemistry.

5. Magnesium ions stimulate the variable fluorescence with both 638 nm and 722 nm light to a similar extent, which argues against the concept of magnesium ion interrupting energy transfer between Photosystem II and Photosystem I.

INTRODUCTION

The conventional view of energy migration within the photochemical apparatus is from light-harvesting chlorophyll or accessory pigment molecules to trapping or reaction-centre chlorophyll species, with absorption bands situated at longer wavelengths. This so-called "downhill" energy transfer is thought to proceed by a Perrin–Forster mechanism of resonance transfer and is dependent on the degree of overlap between the fluorescence emission bands of the light-harvesting pigments and the absorption band of the trapping species [1]. However, it was pointed out by Clayton [2] that it is the transfer into a metastable state at the reaction centre which may be the dominant factor regulating energy trapping, and compared to this, the overlap

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, phenazinemethosulfate.

integrals may not be of paramount importance. Experimental evidence for this comes from the purple bacterium *Rhodopseudomonas* sp. NHTC 133, in which the reaction centre pigment (P 985) has its absorption peak at shorter wavelengths than the main absorption band at 1012 nm. More recently Ben-Hayyim and Malkin [3] working with lettuce chloroplasts have demonstrated that the far-red light (715 and 724 nm) was effective in reducing Q, the primary acceptor to Photosystem II, as shown by fluorescence induction, measured at 685 nm. The quantum efficiency of this process was shown to be approximately half that of shorter wavelength red light, and they considered that this probably represented absorption by Photosystem II of the far red light, which was transferred energetically "uphill" to the Photosystem II trap.

In this communication we confirm the observation of Ben-Hayyim and Malkin with *Euglena* chloroplasts and present evidence that this appears to represent an energy transfer from Photosystem I to Photosystem II. The effect of magnesium ions is also investigated in this system, and appears inconsistent with the often held view that divalent cations interrupt the spillover of energy from Photosystem II to Photosystem I [4, 5, 6].

MATERIALS AND METHODS

Chloroplasts were prepared from late logarithmic or early non-logarithmic phase *Euglena gracilis*, cultured as previously described [7]. Cells were broken in the French press at 1000 lb/inch^2 in a tricine buffer (0.05 M, pH 7.8) containing 0.1 M NaCl and 0.2 M sucrose. The pressurate was centrifuged briefly at approx. 300 g to remove unbroken cells and the supernatant was spun for 5 min at $1500 \times g$ to precipitate the chloroplasts which were resuspended and washed once in a tricine buffer (0.05 M, pH 7.6) containing NaCl 10 mM and sucrose 0.4 M and finally resuspended in the same buffer. All reactions were performed in this buffer.

Fluorescence induction experiments were made in an instrument to which an oscilloscope equipped with memory storage was attached. The photomultiplier (EMI 9659 Q/B) was situated at 90 ° to the exciting beam. Fluorescence was excited either through a combination of Balzers 638 nm and Schott 640 nm filters resulting in maximal transmission at 638 nm (half-band width, 8 nm), or through a Balzers 722 nm filter (half-band width, 10 nm). Measurement was through a combination of Balzers 682 nm and Schott 678 nm filters, resulting in maximal transmission at 681 nm (half-band width, 8 nm). It is unlikely that any scattered light reached the photomultiplier, as no signal was detected when the chloroplast particles were resuspended in buffer, after chlorophyll had been extracted by treatment with 80 % acetone. Methylviologen reduction was measured indirectly as oxygen uptake in a Clark-type electrode. Chlorophyll was measured according to Arnon [8].

RESULTS

In Fig. 1 oscilloscope tracings of the fluorescence induction kinetics with 722 nm and 638 nm exciting light are shown. Qualitatively they appear similar, both with the typical biphasic induction curve, though the ratio $F_\infty - F_0 / F_\infty$ (F_∞ = maximum fluorescence; F_0 = non-variable fluorescence) is lower for 722 nm-light, indicating a relatively lower variable component. From the data in Table I, where

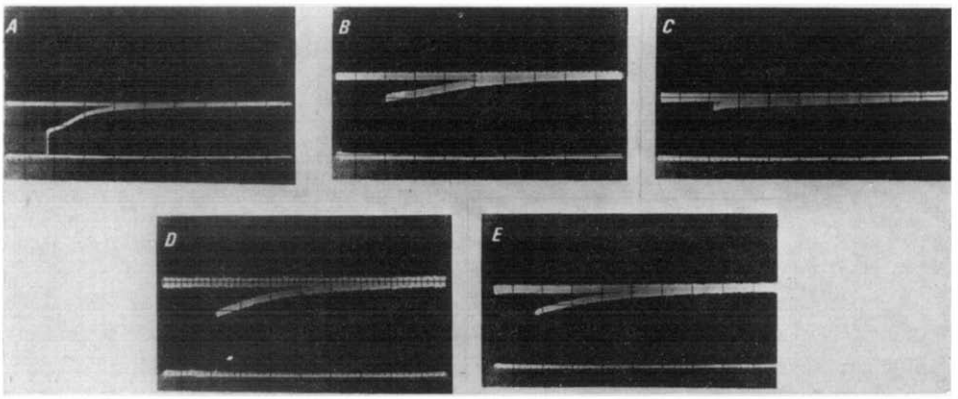

Fig. 1. Oscilloscope tracings of fluorescence induction kinetics with *Euglena* chloroplasts measured at 638 nm (1500 ergs · cm^{-2} · s^{-1}) and 722 nm (20 000 ergs · cm^{-2} · s^{-1}) exciting lights. Chlorophyll content was 10 μg/ml. (A) 638 nm light, no additions. Horizontal scale; 1 s/division, vertical scale; 1 division = 20 mV. (B) 722 nm light, no additions. Horizontal scale, 1 s/division; vertical scale, 1 division = 5 mV. (C) 722 nm light, plus methylviologen 1 mM. Scales as for B. (D) 722 nm-light, plus DCMU 10^{-5} M. Horizontal scale, 0.5 s/division; vertical scale, 1 division= 5 mV. (E) 722 nm-light, DCMU plus methylviologen added. Scales as for D.

the exciting light intensity was manipulated to permit an approximately equal absorption of energy, it can be seen that 638 nm light excited a much greater fluorescence emission than 722 nm-light, with the difference most pronounced in the variable component. It is not possible here to calculate quantum or relative quantum efficiencies following the approach of Malkin and Kok [9], as their equations do not hold for these experiments owing to the low intensities of light absorbed which, therefore, do not fully reduce Q.

TABLE I

Fluorescence parameters, measured at 681 nm with exciting light of 638 nm (3000 ergs · cm^{-2} · s^{-1}) and 722 nm (20 000 ergs · cm^{-2} · s^{-1}). F_0 = nonvariable fluorescence. F_∞ = maximal fluorescence. Chlorophyll: 2.5 μg/ml. Results are the mean values of 3 experiments.

	638 nm	722 nm
F_0	2.4	1.05
F_∞	5.2	1.8
$(F_\infty - F_0)/F_\infty$	0.54	0.42

To investigate whether this effect of 722 nm-light was due to absorption within Photosystem II or to energy transfer from Photosystem I to Photosystem II, methylviologen was included in the reaction medium. The low-potential dye methylviologen is known to accept electrons from Photosystem I. If Photosystem I-absorbed energy is transferred to Photosystem II, treatment with methylviologen should reduce this by stimulating Photosystem I photochemistry, thereby decreasing the number of quanta available to Photosystem II. Data from such experiments are given in Fig. 1, Table II

TABLE II

The effect of methylviologen (0.7 mM) on the fluorescence parameters in the absence and presence of DCMU (10^{-5} M). The exciting beam was 722 nm (20 000 ergs · cm^{-2} · s^{-1}) and the measuring wavelength was 681 nm. Chlorophyll content was 10 μg/ml. Results are the mean values of 5 experiments.

	Control	Methylviologen
F_0	2.0	1.7
F_∞	2.8	2.0
$F_\infty - F_0/F_\infty$	0.28	0.15
	DCMU	DCMU+methylviologen
F_0	2.0	1.9
F_∞	3.1	2.75
$(F_\infty - F_0)/F_\infty$	0.35	0.31

TABLE III

Effect of methylviologen (0.8 mM) on the fluorescence parameters in the presence of DCMU (7 μM) and the uncouplers NH_4Cl (3 mM) and gramicidin (4 μM). Fluorescence was measured at 681 nm and excited at 722 nm (20 000 ergs · cm^{-2} · s^{-1}). Chlorophyll content was 8 μg/ml. Results are the mean values of 4 experiments.

	DCMU	DCMU+methylviologen
Minus uncoupler		
F_0	1.47	1.35
F_∞	2.42	2.0
$(F_\infty - F_0)/F_\infty$	0.39	0.32
Plus NH_4Cl		
F_0	1.4	1.3
F_∞	2.3	1.8
$(F_\infty - F_0)/F_\infty$	0.39	0.28
Plus gramicidin		
F_0	1.35	1.30
F_∞	2.3	1.90
$(F_\infty - F_0)/F_\infty$	0.40	0.32

and Table III, where it can be seen that both in the presence and absence of DCMU, methylviologen reduced the variable fluorescence component, i.e. that component due to Q reduction. This effect displayed a similar methylviologen concentration dependency as methylviologen-stimulated electron transport (with the 2,6-dichlorophenolindophenol-ascorbate-cytochrome 552 donor system in the presence of DCMU); both effects saturated at 150–200 μM methylviologen. Typically this quenching effect was greatest in the absence of DCMU, as is expected, when Q may react with the oxidised pool of electron carriers on its reducing side (see Malkin and Kok [9]). Quenching due to this property, however, may not be of as great an importance in *Euglena* as in chloroplasts from other species, as much or all of the cytochrome 552 is washed out during chloroplast preparation, thus impeding electron transport between the photosystems. Additions of cytochrome 552 to such chloroplasts increases electron transport by at least a three-fold factor. However, in

the presence of DCMU it is clear that no electron transport occurred between the photosystems and yet methylviologen was still effective in reducing the variable fluorescence. A similar quenching, though smaller, has been observed with phenazine-methosulfate.

It has been suggested that in higher plants the quenching of fluorescence by phenazinemethosulfate and also by diaminodurene may be due to the development of the phosphorylation high energy state [5, 10–12]. These workers have demonstrated that uncoupling agents reverse this quenching. However, the effect of methylviologen on the variable fluorescence in the *Euglena* system is apparently a different process as it is in no way effected by uncouplers (Table III).

The ability of methylviologen to quench fluorescence should be dependent on the presence of electron flow to this compound from Photosystem I reaction centres, which is in turn dependent on an electron donor system. In the presence of DCMU water does not fulfill this role. However, in these chloroplasts there is a pool of an endogenous electron donor which permits the light-dependent reduction of methyl-viologen at low rates (6 μmoles/mg chlorophyll/h has been observed with 15 000 ergs · $cm^{-2} \cdot s^{-1}$ of 722-nm light incident) in the presence of high concentrations of DCMU (10^{-5} M), as determined by measurement with an oxygen electrode. The size of this pool is approximately 2 electron equivalents per chlorophyll.

When *Euglena* is placed in the dark under dividing conditions, chlorophyll levels decline, largely by a process of dilution through division [13]. We have noted that there is no relative change in 638 nm or 722 nm absorbing chlorophylls during a fourfold dilution of chlorophyll by this process, contrary to the greening situation [13]. It was, therefore, possible to investigate the effect of chlorophyll dilution on the Photosystem I to Photosystem II energy transfer. In Fig. 2 it can be seen that upon chlorophyll dilution the ratio $F_\infty - F_0 / F_\infty$ declined with both 638 nm and 722 nm, as is expected on the basis of the decreased efficiency of energy transfer. However, this was much more marked with 722 nm-light, suggesting that the Photosystem I to

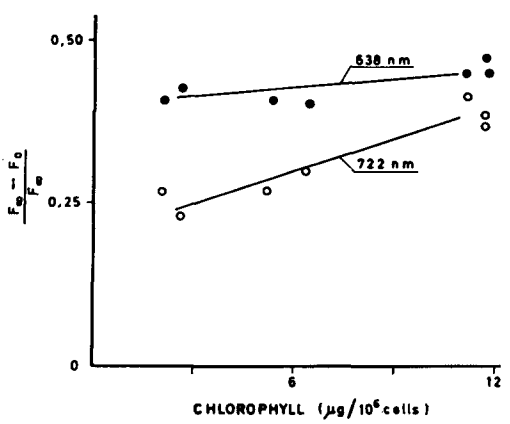

Fig. 2. Ratio of variable ($F_\infty - F_0$) to maximal (F_∞) fluorescence yield as a function of chlorophyll content of *Euglena* cells, placed in the dark to divide. Fluorescence was excited with either 638 nm (1500 ergs · $cm^{-2} \cdot s^{-1}$) or 722 nm (20 000 ergs · $cm^{-2} \cdot s^{-1}$) light and measured at 681 nm. The chlorophyll content was 4 μg/ml. Results are from 2 experiments.

TABLE IV

Effect of $MgCl_2$ (5 mM) on the fluorescence parameters, measured at 681 nm and excited with 638 nm (3000 ergs · cm^{-2} · s^{-1}), and 722 nm (20 000 ergs · cm^{-2} · s^{-1}) light. Chlorophyll content was 5 μg/ml. Results are the mean values of 3 experiments.

	638 nm		722 nm	
	Minus $MgCl_2$	Plus $MgCl_2$	Minus $MgCl_2$	Plus $MgCl_2$
F_0	4.2	4.4	1.9	2.1
F_∞	8.6	13.6	3.3	5.2
$F_\infty - F_0$	4.4	9.2	1.4	3.1

Photosystem II transfer was much more strongly effected than energy transfer within Photosystem II itself.

In Table IV data are presented for an experiment in which the effect of magnesium ions on the fluorescence excited by 722 nm-light is compared with that excited by 638 nm-light. As previously reported for *Euglena* chloroplasts [7] and higher plant chloroplasts [4, 5] the magnesium ion stimulation of the fluorescence yield is largely, if not entirely, due to the variable component, and was approximately doubled by magnesium ions with either 638 nm- or 722 nm-light.

DISCUSSION

In this paper we have shown that energy absorbed at long wavelengths (722 nm) in *Euglena* chloroplasts is transferred "uphill" to fluoresce at shorter wavelengths. Furthermore this light is able to perform the typical Photosystem II activity of reducing Q, as indicated by the variable fluorescence rise, in a similar manner to shorter wavelength light, and in a reaction which is decreased by both methylviologen and PMS even in the absence of electron transport from Photosystem II to Photosystem I. This effect of methylviologen is not reversed by uncoupling agents which indicates that the quenching mechanism differs from that previously described [5, 10–12], which seems to be related to the formation of the high energy phosphorylating state. We therefore feel that this quenching is best explained as being due to a stimulation of Photosystem I photochemistry, thereby reducing the energy available for transfer to Photosystem II and thus lowering the extent of Q reduction. The possibility that this is a non-physiological quenching, brought about by a direct effect of methylviologen on excited state chlorophyll seems unlikely to us for a number of reasons. Firstly, both Photosystem I reduction of methylviologen and the fluorescence quenching effect in the presence of DCMU, display a similar methylviologen concentration dependency. Secondly, methylviologen is not expected to interact directly with chlorophyll owing to its hydrophilic nature. Thirdly, the effect on fluorescence quenching is mainly on the variable component. The much smaller effect on the non-variable component is probably due to the expected quenching of Photosystem I fluorescence. This energy transfer appears to be quite strongly dependent on chlorophyll concentration in the lamellae, as it is substantially reduced when chlorophyll is diluted during division in the dark. To our knowledge this is the first time that strong evidence has been put forward for the transfer of energy

from Photosystem I to Photosystem II.

It should be pointed out that we have noticed the quenching effect of methylviologen on the variable fluorescence to be of a similar magnitude with 638 nm-light as with 722 nm-light. This is consistent with the observation that energy absorbed at 638 nm is almost as effective as 708 nm light in mediating the Photosystem I reduction of methylviologen in Euglena [7]; an observation interpreted to indicate a very efficient Photosystem II to Photosystem I "spillover" in *Euglena.*

Recently several groups [5, 6] have supported the idea first put forward by Murata [4] that magnesium and other divalent cations inhibit the "spillover" of energy from Photosystem II to Photosystem I, and thus increase the fluorescence yield. However, in previous publications on this subject [7, 14] we have been unable to agree with this interpretation, mainly on the basis that in *Euglena* the Photosystem I reduction of methylviologen with Photosystem II-enriched light is not sensitive to magnesium ions while there is a large fluorescence stimulation. Secondly, the stimulation of Photosystem II electron transport by magnesium ions has a different magnesium ion concentration requirement than the fluorescence response [14], and so should not be used to support the "spillover interruption" hypothesis, as has been done [4, 6]. The data presented here are also not favourable to this concept, as the fluorescence stimulated by Photosystem I-absorbed light is just as responsive to magnesium ions as that excited by Photosystem II-absorbed light.

The observation by Murata [4] and recently confirmed by Gross and Hess [15], that divalent cations decrease "Photosystem II-fluorescence" and increase "Photosystem I-fluorescence" at liquid nitrogen temperature is frequently used to support the "spillover interruption" hypothesis. It should be pointed out however, that a similar reduction of long wavelength fluorescence has not been demonstrated at room temperature [5, unpublished observations] when the magnesium ion-stimulation of short wavelength fluorescence is clearly seen. We, therefore, feel that the fluorescence emission spectra information cannot be used to support the "spillover interruption" hypothesis of divalent cation-induced fluorescence increase, which may be accounted for by the suggestion [14] that divalent cations divert energy from non-radiative dissipative processes to fluorescence.

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REFERENCES

1 Clayton, R. K. (1965) Molecular Physics of Photosynthesis, Blaisdell Publishing Company, New York
2 Clayton, R. K. (1966) Proc. 2nd West Eur. Conf. on Photosynth. Ad. Donker, p. 105
3 Ben-Hayyim, G. and Malkin, S. (1972) 2nd Int. Congr. Photosynth. Res. (Forti, G., Avron, M. and Melandri, B. A. eds), p. 61, Junk Publishers, The Hague
4 Murata, N. (1969) Biochim. Biophys. Acta 189, 171
5 Mohanty, P., Braun, B. Z. and Govinjee (1973) Biochim. Biophys. Acta 292, 459
6 Briantais, J. M., Vernotte, C. and Moya, I , (1973) Biochim. Biophys. Acta 329, 530
7 Jennings, R. C. and Forti, G. (1974) Plant Sci. Lett. 3, 25

8 Arnon, D. I. (1949) Plant Physiol. 24, 1
9 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413
10 Murata, N. and Sugahara, K. (1969) Biochim. Biophys. Acta 189, 182
11 Wraight, C. A. and Crofts, A. R. (1970) Eur. J. Biochem. 17, 319
12 Cohen, W. S. and Sherman, C. A. (1971) FEBS Lett. 16, 319
13 Brody, M. (1968) in The Biology of Euglena (D. E. Buetow, ed.), p. 215, Academic Press, New York
14 Jennings, R. C. and Forti, G. (1974) Biochim. Biophys. Acta 347, 299
15 Gross, E. L. and Hess, S. C. (1973) Arch. Biochem. Biophys. 159, 832