Antagonistic Relationships between Electron Transport and P_{700} in Chloroplasts and Intact Algae

Hans J. Rurainski

Lehrstuhl für Biochemie der Pflanze der Universität Göttingen

The effects of divalent salts and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) upon nicotinamide adenine dinucleotide phosphate (NADP) reduction and P_{700} in isolated chloroplasts are described and compared with the influence of DCMU on oxygen evolution and P_{700} in intact cells.

Most experiments were carried out with a steady-state relaxation spectrometer. A kinetic mechanism for the estimation of P_{700} fluxes is proposed and experimentally tested. Good agreement between theory and experiment was found.

Concurrent measurements of P_{700} and NADP reduction revealed two antagonisms: addition of divalent cations caused an increase in the yield of NADP reduction and a decrease in the yield of P_{700}. Conversely, in the presence of Mg^{2+} low concentrations of DCMU decreased the yield of NADP reduction and increased the yield of P_{700}. Aging of the chloroplasts at 30 °C exerted a similar effect.

With far-red actinic light, Mg^{2+} stimulated the yield of NADP reduction without affecting the flux. Also, in the absence of Mg^{2+}, DCMU inhibited both reactions although P_{700} required a higher herbicide concentration for fractional inhibition than NADP reduction.

In the presence of Mg^{2+}, chloroplasts resembled intact algae in which a high rate of oxygen evolution was accompanied by little P_{700} turn-over. Titration with DCMU decreased the rate of photosynthesis and increased P_{700} flux.

On the whole, the data suggest that P_{700} relaxing in 20 msec is not directly involved in linear electron transport.

Introduction

The currently discussed electron transport scheme of photosynthesis includes P_{700} as the reaction center of photosystem I (for details see a recent review [1]). On the basis of its position in the scheme, certain predictions concerning the turnover rate of this pigment in relation to NADP reduction and oxygen evolution may be made: ideally, the rate of electron transport through the pigment (the flux) should be equal to the rate of either oxygen evolution or NADP reduction. A higher flux than the terminal reaction rate is possible if one assumes that not all electrons passing through the pigment arrive at the site of the terminal reaction but perhaps participate in a "cyclic" electron transport. A third possibility, however, *viz.* a flux smaller than the rate of NADP reduction would be inconsistent with the scheme.

Previous studies on the relationship between linear electron transport and the turn-over of P_{700} yielded different results for intact cells than for isolated chloroplasts. For example, excitation of several red and blue-green algal species with short-wavelength light resulted in high rates of oxygen evolution but a small turn-over of P_{700} [2-4]. With isolated chloroplasts, on the other hand, P_{700} flux was found to be nearly equal to or to exceed the rate of NADP reduction [3, 5]. Also, from experiments with flashed saturating excitations, it has been concluded that P_{700} receives most of the electrons from light reaction II [6]. Only the addition of Mg^{2+} to chloroplasts excited with moderate light intensities which resulted in an increase of the quantum yield of NADP reduction and a concomitant decrease in the P_{700} yield [7] led to a resemblance in terms of these parameters between chloroplasts and algae.

A second difference was observed in studies with the herbicide DCMU. The inhibition of photosynthesis in intact algae either caused no change or increased the signals at 700 nm [3, 8]. In isolated chloroplasts, however, both P_{700} and NADP reduction were inhibited in a similar fashion and a DCMU insensitive P_{700} was observed only in the

Abbreviations: NADP, Nicotinamide adenine dinucleotide phosphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, phenazine methosulfate.

presence of PMS [9]. Thus, the relationship between P_{700} and photosynthesis appeared to be antiparallel in intact cells but parallel in chloroplasts.

The experiments reported here extend our previous observations on the role of Mg^{2+} (ref. 7, 10). We will show that several other ions can replace this cation in causing antagonistic behaviour between NADP reduction and P_{700} flux. In addition, we will report data on the inhibitory effect of DCMU in chloroplasts and intact cells which indicate a further antiparallelism between the two reactions. Mg^{2+} appears to play a key role also in this instance.

In our interpretation of the data, we conclude that the pigment with a relaxation time of approximately 20 msec. cannot be involved in a linear electron transport from water to NADP. A recently reported, fast relaxing P_{700} will be considered.

Materials and Methods

The preparation of chloroplasts and the reaction conditions have been described previously [10]. *Chlorella pyrenoidosa* was obtained from synchronous cultures in the 22nd hour of the synchronous cycle, *i.e.* shortly after release of the autospores [11]. *Anacystis nidulans* was grown at 30 °C in the medium of Hecker [12]. In all cases, the experiments were carried out in the respective growth medium of the cells.

Short wavelength illumination was obtained by filtering the actinic light through a broad-band interference filter transmitting between 530 and 645 nm; the intensity at the cuvet was 2×10^4 erg $(cm^2 sec)^{-1}$. For far-red light, a narrow-banded interference filter whose transmission centered at 696 nm was used. The intensity was 1.45×10^3 ergs $(cm^2 sec)^{-1}$.

Oxygen evolution was measured with a Clark-type Pt-AgCl electrode (Rank Bros., Bottisham, England) whose temperature was maintained at 25 °C.

NADP reduction and P_{700} were measured with a steady-state relaxation spectrophotometer which has been described elsewhere [13]. In this instrument, the sample is illuminated with modulated actinic light of a given frequency. NADP reduction was followed by the light-dependent, time-averaged change of absorbance at 340 nm. The extinction coefficient used was 6.25 $mM^{-1} cm^{-1}$.

P_{700} was measured by absorbance changes at 700 nm. These absorbance changes are modulated at the some frequency as the actinic light but are shifted in phase. The signals are analyzed in a phase-sensitive lock-in amplifier and an electrometer

and provide a measure of the time-averaged transmission as well as the amplitude and the phase angle of the absorption change. The amplitude is proportional to the concentration of P_{700} which turns over, while the phase angle may be expressed in terms of a "relaxation time", the time required for the dark decay of the pigment. Provided that the illumination is low enough so that the amplitude is linearly proportional to the intensity and that the dark reduction is a first-order reaction, the relaxation time may be expressed as a rate constant. The flux, the product of the concentration term and the rate constant, can then be calculated according to

$$v_{700}(\mu equiv./mg\ Chl\ h) = \frac{m\ k\ A}{\varepsilon \cos \varphi}$$

where ε is the extinction coefficient of P_{700} (taken as $65\ mM^{-1} cm^{-1}$ (ref. 14) and m is a collection of constants which gives the flux the dimension of $\mu equiv/mg\ Chl\ h$. $\cos \varphi$ derives from the measurement and corrects the attenuation of the signal due to the modulation.

In order to illustrate the derivation of this equation and to show that flux calculations are reasonable for P_{700}, we shall give a brief kinetic treatment of, and experimental justification for, the model underlying the calculation.

In the photochemical reaction

$$p + h\nu \underset{k}{\overset{\Phi}{\rightleftharpoons}} p^*.$$
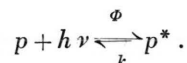
p and p^* are dark and light-induced forms of P_{700} and Φ and k the rate constants for the oxidation and reduction of the pigment.

The rate equation for this reaction is

$$dp^*/dt = I\ \Phi(p_T - p^*) - k\ p^* \qquad (1)$$

with p_T equal to the concentration of all forms of p. Due to the time-dependence of both I and p^*, the equation is non-linear but is linearized for $p^* \ll p_T$, a condition which holds for small perturbations, *i.e.* at low light intensities. Thus, neglecting p^* in comparison to p_T, the rate equation reads

$$dp^*/dt = I\ \Phi\ p_T - k\ p^*. \qquad (2)$$

For a perfect sinusoidal modulation at angular frequency $\omega = 2\pi f$, the applied actinic light intensity varies with time according to

$$I(t) = I_0(1 + \sin \omega t) \qquad (3)$$

where I_0 is the average intensity.

The absorption changes caused by this actinic light must also be harmonic, *i. e.* p^* oscillates at the same frequency but undergoes a phase shift due to

a finite reaction time. Let the solution be

$$p^*(t) = p_0^* + p_\omega^* \sin(\omega 4t - \varphi) \qquad (4)$$

where p_0^* is the time-averaged concentration of oxidized pigment and p_ω^* is one-half the amplitude at the experimental modulation frequency. The angle φ describes the phase shift between the actinic light and the absorption change.

In practice, the actinic light is less than a perfect sinusoid. Hoch[13] pointed out, however, that this case, if treated more rigorously as a Fourier series of sine waves resulted in only small gain of accuracy.

Differentiation of (4) yields

$$dp^*/dt = \omega\, p_\omega^* \cos(\omega t - \varphi). \qquad (5)$$

Substitution of (3), (4) and (5) into Eqn (2) results in

$$p_0^* = p_\omega^*/\cos\varphi \qquad (6)$$

and

$$k = \omega/\tan\varphi. \qquad (7)$$

The time-averaged electron flux through P$_{700}$ is then

$$v_{700} = k\, p_0^* = \omega\, p_\omega^*/\sin\varphi.$$

The actual measurement involves the determination of the in-phase and out-of-phase (with the actinic light) amplitudes of the modulated absorption changes. These have values of $p_\omega^* \cos\varphi$ and $p_\omega^* \sin\varphi$, respectively and are the outputs of the lock-in amplifier. Using Eqn (6) and well-known trigonometric identities, their magnitude according to the model have been calculated as a function of modulation frequency and were plotted as a drawn-out curve in Fig. 1. As the modulation frequency increases the signal amplitude decreases. This is a reasonable behavior because for a given relaxation

time a progressively smaller portion of the excited pigment returns to the dark state as the frequency of exciting "flashes" increases. Experimental measurements of these parameters with chloroplasts in the presence of methyl viologen were normalized to the theoretical curve and are represented by the dots in Fig. 1. Quite clearly, theoretical and experimental results closely fit.

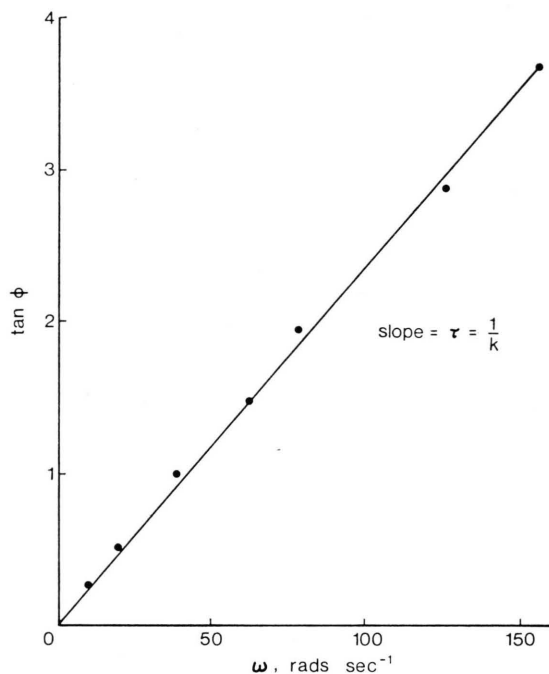A second test of the model is shown in Fig. 2. Eqn (7) requires a linear dependance of than φ on

Fig. 2. Dependence of tan φ on the frequency, test of Eqn (7). Experimental conditions as in Fig. 1.
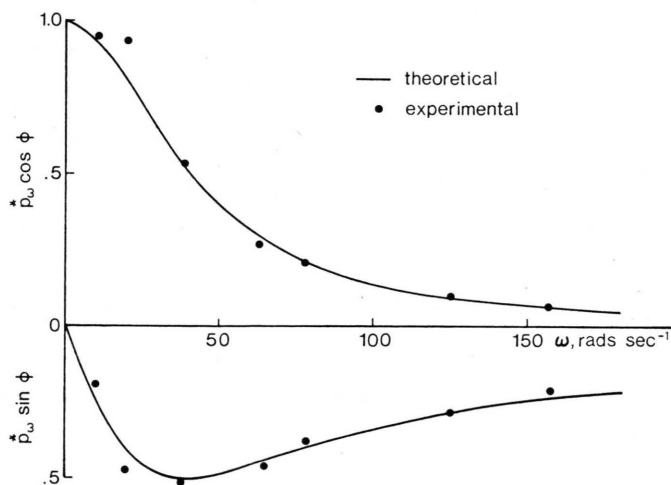

Fig. 1. Frequency dependence of recorded signals; test of Eqn (6). The reaction mixture contained 50 mM tris-HCl buffer, pH 7.5, 400 mM sucrose, 20 mM NaCl, 1 mM methyl viologen and 23 μg chlorophyll per ml. White actinic light. The solid lines are plots derived from Eqn (5), the points are experimentally measured and normalized outputs of the lock-in amplifier.

the modulation frequency. This means, that the relaxation time or the rate constant are independent of the modulation frequency used to measure it. (tan φ is obtained from the ratio of the measured amplifier outputs: $\tan \varphi = p_\omega^* \sin \varphi / p_\omega^* \cos \varphi$). As demonstrated in Fig. 2, the requirement is met, although under some conditions the test could not be carried out. For example, upon addition of cations to chloroplasts, the signals became so small that it was difficult to determine their frequency dependance. Nevertheless, when tested, the experimental results agreed closely with the theoretical requirement.

Lastly, we compared the rate constant for relaxation as a function of signal amplitude. Eqn (7) predicts that the rate constant is independent of the concentration of reacting pigment. On the other hand, the rate constant for the reduction of P_{700} in the presence of PMS was found to be a second order reaction [15] and should increase with increasing substrate concentration. Therefore, we compared the relaxation of the pigment in Hill reactions with its relaxation in the presence of PMS. For this purpose, the signal amplitude was varied through a variation of the actinic light intensity. As shown in Fig. 3, the calculated k in the presence of both
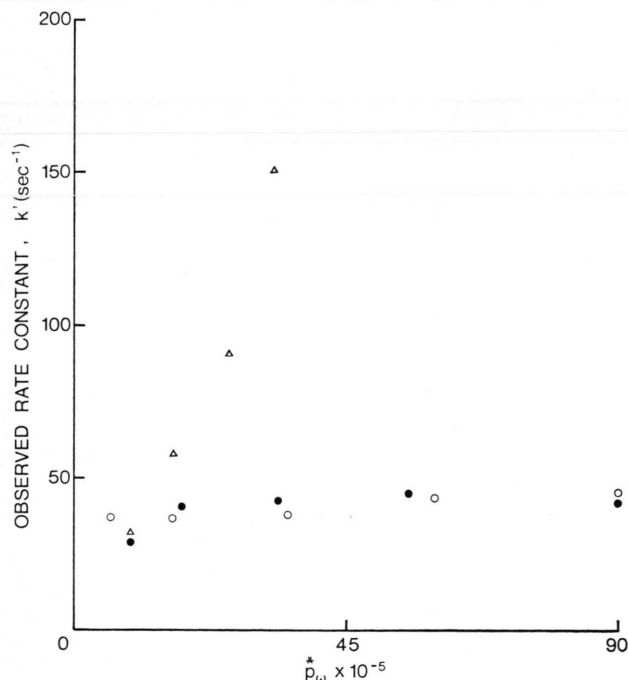

Fig. 3. Comparison of observed rate constants in first- and higher-order reactions of P_{700}. Reaction medium as in Fig. 1. Substrates: solid circle; 0.5 mM NADP with 40 μg per ml Ferredoxin; open circles, 0.5 mM Fe(CN)$_6^{3-}$; triangles, 10 μM PMS. Modulation frequency 6.25 cps, white actinic light.

NADP and K-ferricyanide is independent of the extent of P_{700} turn-over. With PMS, the order of the relaxation process is, indeed, greater than one so that the above mechanism cannot be applied in this instance.

Thus, the relaxation technique described here can provide quantitative information about the kinetic behavior of P_{700}. One of the demands for the measurements is that they be made at low light intensities with small displacements of the excited pigment from the dark state. The magnitude of the signals observed under these conditions is very small. Fortunately, the lock-in technique provides excellent signal/noise ratios because of extreme narrow-banding of the amplifier.

Results

In previous reports, we described the effect of $MgCl_2$ upon the yields of NADP reduction and P_{700} flux in short-wavelength actinic light [7, 10]. Concomitant with an approximately two-fold increase in the former, we observed a large decrease in the latter. In order to further characterize this effect, it was of interest to determine whether $MgCl_2$ was specific in its action. The work of Murata [16] and Li [17] had already shown that a variety of different salts influenced several other chloroplast reactions.

Table I shows that Mg-sulfate and Mg-nitrate were equally as effective as the chloride in raising the rate of NADP reduction and decreasing the flux. Thus, the observed changes are presumably caused by the cation. Similar results were obtained with Ca^{2+}, Mn^{2+} and Na^+, although a more than twentyfold higher concentration of the monovalent than of the divalent ions was required.

Table I. Effect of various salts upon P_{700} turn-over and NADP reduction. The reaction mixture contained 15 mM tricine, pH 7.5, 50 mM sucrose, 20 mM NaCl, 0.25 mM NADP; also per ml: 20 μg chlorophyll, 30 μg ferredoxin. Modulation frequency: 6.25 cycles/sec; short-wavelength actinic light.

Salt [mM]	v_{340} [μequiv./mg Chl h]	v_{700}	$\dfrac{\Delta v_{700}}{\Delta v_{340}}$
Control	14.0	43.0	—
MgCl$_2$, 5	31.3	7.5	2.05
MnCl$_2$, 5	30.1	9.6	2.07
CaCl$_2$, 5	32.9	7.3	1.89
NaCl, 100	26.2	19.0	1.97
MgSO$_4$, 5	31.0	8.0	2.06
Ca(NO$_3$)$_2$, 5	28.1	11.1	2.26

As in preiously reported experiments [7], the ratio between the decrease in P$_{700}$ flux (Δv_{700}) and the increase in the NADP reduction rate (Δv_{340}) was nearly a constant, independent of the cation causing the change or the magnitude of the change. With an extinction coefficient of 65 mM^{-1} cm^{-1} used here, the ratio was close to two.

In contrast to this stoichiometry routinely observed with fresh chloroplasts and in short-wavelength light, different results were obtained with far-red illumination. Comparative data are shown in Table II. In 696 nm actinic light, addition of Mg^{2+} still increased the rate of NADP reduction by a similar factor as in short-wavelength light, but the flux through P$_{700}$ did not change. Thus, the antagonism between the two reactions does not hold in this case.

Table II. Comparison of Mg^{2+} action in two colors of light. Experimental conditions as in Table I, except 13 μg/ml chlorophyll. 5 mM Mg^{2+} was added where indicated. Modulation frequency: 6.25 cycles/sec. All rates are expressed as μ equiv./mg Chl h.

| | v_{340} | | v_{700} | |
Actinic light	$-$Mg^{2+}	$+$Mg^{2+}	$-$Mg^{2+}	$+$Mg^{2+}
short-wavelength	12.0	25.5	30.8	3.3
far-red	3.8	7.7	12.8	14.0

The effects of Mg^{2+} were quite reproducable and independent of whether chloroplasts were isolated from spinach or peas. However, in the winter months when experiments were carried out with spinach from the local market, the magnitude of the P$_{700}$ decrease often varied considerably although an increase in NADP reduction was observed. Subsequent use of grenhouse-grown peas largely eliminated the variation. This observation suggested that the effect could be related to the age of the plant material. In order to test this idea, we isolated chloroplast from young leaves and incubated them at 30 °C in the isolation medium for increasing periods of time and then assayed at room temperature.

Fig. 4 shows the response of the chloroplasts to this ageing. A the incubation progressed, both P$_{700}$ and NADP reduction were inhibited when Mg^{2+} was absent. In the presence of the salt, v_{340} also decreased but the flux through P$_{700}$ first increased from its low value to a maximum and then was gradually inhibited. During the entire incubation period, addition of Mg^{2+} resulted in an approximate
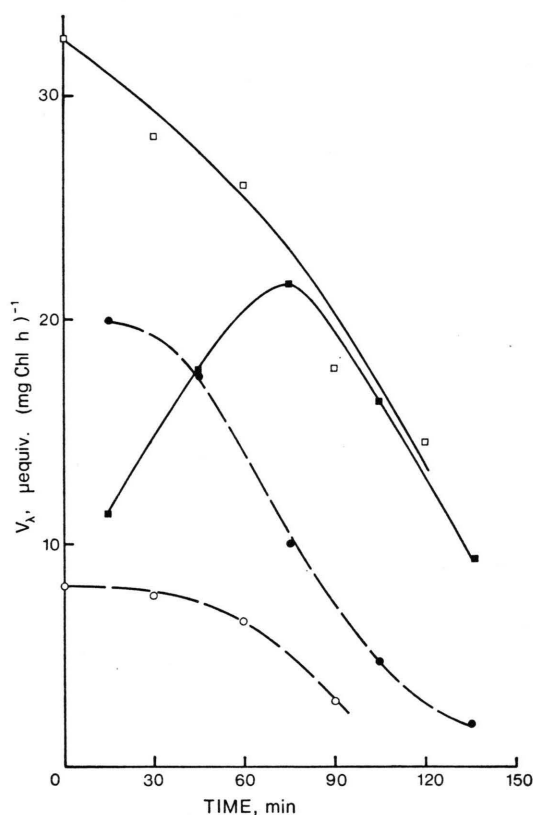

Fig. 4. Effect of Mg^{2+} in aged chloroplasts. The reaction mixture contained 15 mM tricine buffer, pH 7.5, 50 mM sucrose, 20 mM NaCl, 2.5 mM NH$_4$Cl, 0.25 mM NADP; also per ml: 12 μg chlorophyll, 30 μg ferredoxin. Solid lines: P$_{700}$ flux; dashed lines: NADP reduction. Open symbols: $-$ Mg^{2+}; closed symbols: $+$5 mM Mg^{2+}. Modulation frequency 6.25 cps.; short-wavelength actinic light.

doubling of the NADP reduction rate; however, the salt became increasingly less effective in suppressing P$_{700}$. After long incubation times almost no apparent change in v_{700} was observed. Prior to a closer analysis of these data, we present further experiments concerning the response of chloroplasts to DCMU which, in many respects, yielded similar results.

The herbicide DCMU has long been known as a powerful inhibitor of photosynthetic electron transport. Fig. 5 shows that in the absence of Mg^{2+}, both P$_{700}$ and NADP reduction were totally inhibited if high enough concentrations of this reagent were added. However, for a given degree of partial inhibition, the terminal reaction required considerably lower intermediate concentrations than the pigment. In order to emphasize this point, the rates of NADP reduction were additionally normalized
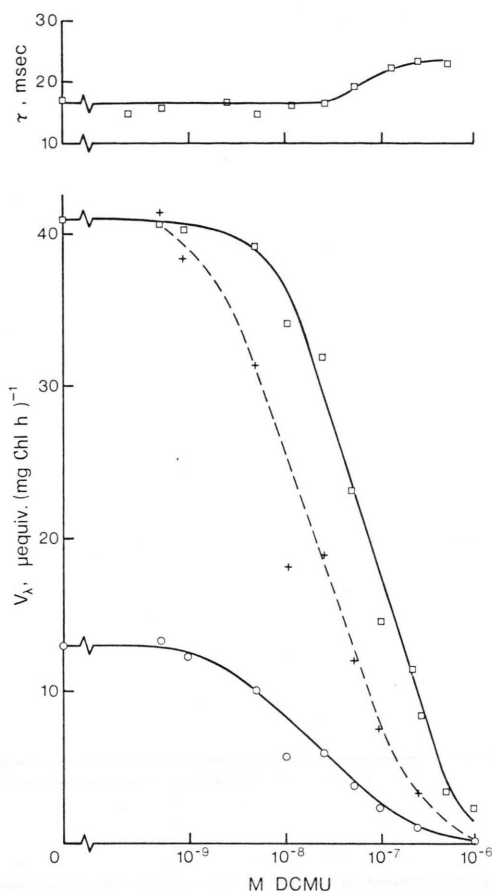
Fig. 5. DCMU inhibition of electron transport in the absence of Mg^{2+}. Experimental conditions as in Fig. 4, except 13 μg per ml chlorophyll. Circles: NADP reduction; squares: P$_{700}$ flux. The crosses connected with the dashed line represent the NADP reduction data normalized to the flux in the control sample.
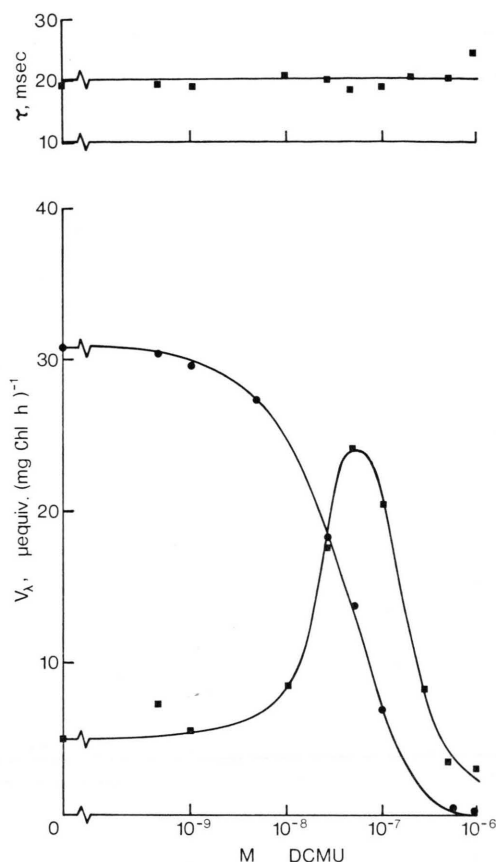

Fig. 6. DCMU inhibition of electron transport in the presence of Mg^{2+}. Experimental conditions as in Fig. 4, except 13 μg per ml chlorophyll. Dots: NADP reduction; Squares: P$_{700}$ flux.

to the flux. It seems that the reduction of NADP can be lowered significantly without effect on P$_{700}$. The magnitude of the difference varied somewhat from one chloroplasts preparation to the next and in some experiments nearly ten-times more DCMU was needed for 50% inhibition of P$_{700}$ than for 50% inhibition of NADP reduction. In no experiment could we find a close correlation in the degree of inhibition for both reactions. The slight increase of the relaxation time of P$_{700}$ from 17 to 23 msec observed in this particular experiment is atypical since usually constant values were found over the entire inhibitor range. This observation indicates an "all-or-nothing" inhibition by DCMU.

A partially different effect of DCMU was observed in the presence of 5 mM Mg^{2+}, i. e. under conditions when the rate of reduction was high and the flux was low (Fig. 6). The shape of the titration curve and the concentration of inhibitor required for 50% inhibition of v_{340} remained approximately the same as in the absence of Mg^{2+}. In contrast, the rate of electron flux through P$_{700}$ first increased from its low level and reached a maximum before being completely inhibited. Thus, as far as these parameters are concerned, addition of DCMU and aging yielded similar results. As suggested above, the relaxation time of P$_{700}$ is constant at all inhibitor concentrations.

The peculiar shape of the P$_{700}$ curve in Figs 4 and 6 is most likely the net results of two overlapping effects. The first one is primarily noticable at low concentration of DCMU or short periods of aging and manifests itself by an increase in the flux. At higher DCMU concentrations or longer periods of aging, the second effect, and inhibition of the flux, sets in and is superimposed upon the first.

At any rate, even if we confine our interpretation to low DCMU concentrations or short incubation times, we again observe an antagonism between the reduction of NADP and P_{700}: as the yield of the former decreased, the yield of the latter increased. We therefore conclude that the P_{700} observed here is not directly involved in the reduction of NADP as postulated by the series scheme of electron transport.

As we and others have previously shown with single measurements of P_{700} in intact algae, addition of DCMU in concentrations which presumably inhibited photosynthesis, raised the 700 nm-signals [2, 8]. In order to determined whether there existed a relation between these data and the experiment of Fig. 6, we titrated suspensions of intact algae with the inhibitor and measured the flux through P_{700} as well as the rate of photosynthesis.
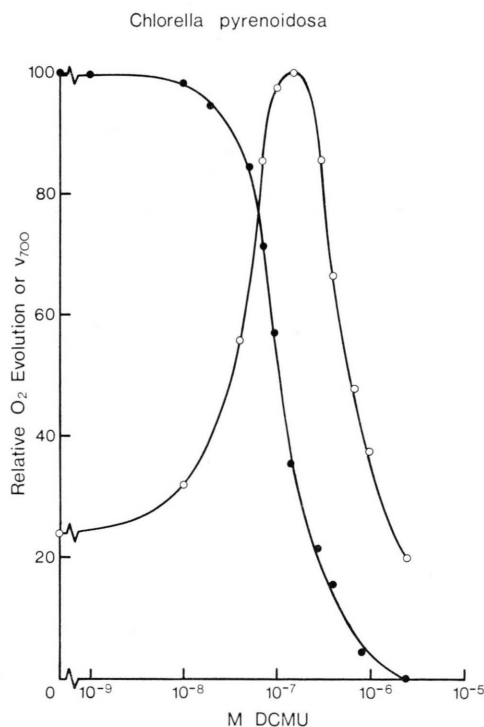
Chlorella pyrenoidosa

Fig. 7. Effect of DCMU on the relative rate of oxygen evolution and relative P_{700} flux of *Chlorella*. The cells were harvested in the 22nd hour of the synchronous growth cycle. Measurements were carried out in the growth medium. Modulation frequency for P_{700}: 10 cps. Short-wavelength actinic light.

Fig. 7 demonstrates the result for the green alga *Chlorella pyrenoidosa* which was taken out of a synchronously growing culture shortly after a com-

plete division cycle. Quite similar data were obtained with batch cultures of *Scenedesmus obliquus* (D_3). With increasing concentrations of DCMU, the relative rate of oxygen evolution descreased, reaching complete inhibition at 10^{-6} M inhibitor. Simultaneously, the relative P_{700} flux followed a similar curve as in the case of chloroplasts after addition of Mg^{2+}. We feel, that again two overlapping and oppositely directed effects produced the peculiar profile of this curve.
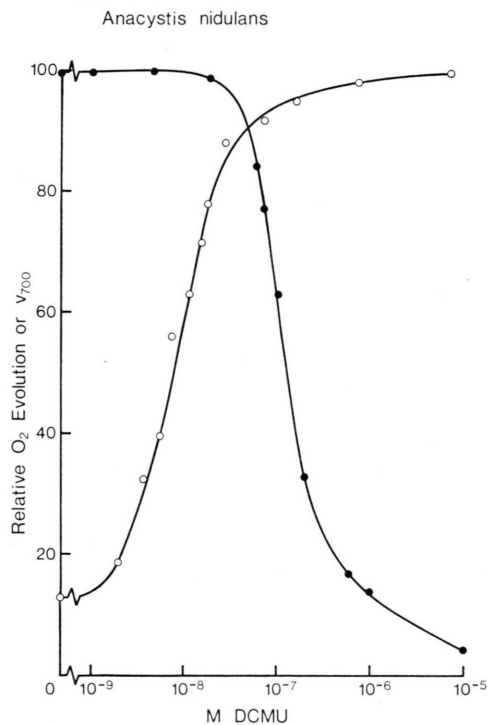
Anacystis nidulans

Fig. 8. Effect of DCMU on the relative rate of oxygen evolution and relative P_{700} flux of *Anacystis*. The cells were grown in batch culture and the rates were measured in the growth medium. Modulation frequency for P_{700}: 10 cps. White actinic light.

A somewhat different result was obtained with the blue-green alga *Anacystis nidulans* (Fig. 8). Again, we observed an antagonistic behavior between photosynthesis and P_{700} flux, but in contrast to the experiments described above, P_{700} turn-over remained large even at the highest concentration of the inhibitor used. Presumably, of the two overlapping reactions postulated for chloroplasts in the presence of Mg^{2+} and the green algae, only the flux-stimulating effect is operative. Further experiments must establish whether this difference can be correlated with any other observable difference between green and blue-green algae.

Discussion

The data reported here extend our previously published experiments concerning the effect of Mg^{2+} upon P_{700} and the reduction of NADP [7, 10]. It is to be emphasized that all discussion remarks refer to P_{700} with a relaxation time in the milli-second range. More rapid relaxations have not been widely observed but one report [18] will be discussed below.

Comparison of P_{700} flux and the rate of NADP reduction shows that the former can easily exceed the latter. Such observations were made in the absence of Mg^{2+} with either short- or long-wavelength light and with aged or DCMU-treated chloroplasts in the absence as well as the presence of Mg^{2+}. This seems to be a rather important point, for if one assumes that P_{700} in excess of v_{340} can "cycle" during the reduction of NADP, one could suppose that the entire turn-over of the pigment is not involved in electron transport to NADP. Of course, as long as v_{700} is greater than v_{340} by a constant factor, the difference could conceivably be due to a false extinction coefficient of P_{700}. The reported flux calculations are based on a value of $65\ mM^{-1}\ cm^{-1}$ measured by Hiyama and Ke [14] and checked by Haehnel [6].

A second conclusion is hardly open to doubts regardless of the extinction coefficient used. In two instances, the results show that the yields in short-wavelength light of the two reactions are inversely related, i. e. a decrease in the yield of v_{700} is accompanied by an increase in the yield of v_{340} and vice versa.

One antagonism has been observed after addition of cations to the reaction which causes a large decline in P_{700} flux and a concomitant doubling of the rate of pyridine nucleotide reduction. In fact, the respective changes in rate occur progressively with increasing cation concentration and are closely related [7]. With the extinction coefficient used here. two equivalents of P_{700} disappear for each equivalent appearing as NADPH. Although this apparent stoichiometry may be entirely fortuitous, it has been calculated for a variety of experimental conditions [7].

A further antagonism between the yields of v_{700} and v_{340} for which the presence of Mg^{2+} is also required was observed after addition of DCMU to, or aging of, the chloroplasts. Both treatments appear to give qualitatively similar results so that the

discussion of one may be representative for the other. Although high concentrations of the inhibitor completely block both reactions, titrations with intermediate concentrations show that the decrease in the yield of NADP reduction is accompanied by a simultaneously increase the yield of the flux. With approximately $10^{-7}\ M$ inhibitor, a maximum flux is reached followed by a further decrease.

A different picture is observed in the absence of Mg^{2+}. Both reactions are progressively inhibited but P_{700} requires considerably more DCMU for any fractional inhibition than NADP reduction. This observation is also difficult to interpret in terms of a linear electron transport between the pigment and the terminal reaction, since then we would expect a close correlation between the two reactions as the inhibitor concentrations increase. Even if we suppose that only part of the electrons measured by the flux are involved in NADP reduction, we would obtain a closer correlation only with additional assumptions. Besides Mg^{2+}, several other divalent as well as monovalent cations in higher concentrations yield the same results. Although these observations provide no obvious explanation of a mechanism of action, they do suggest that the salts exert a physical rather than a chemical effect upon the chloroplasts.

This effect in short-wavelength light is most easily rationalized by assuming that addition of the salt activates non-fluorescing, P_{700}-containing reaction centers and converts them into fluorescing, NADP-reducing centers. This interpretation satisfies the observation that P_{700} decreases and NADP reduction and fluorescence [10, 16, 17, 20] increase. Inactivation of the NADP-reducing centers through aging or with DCMU reverses this phenomenon. With P_{700}, the reversal can be observed directly only with DCMU concentrations up to approximately $10^{-7}\ M$ or relatively short periods of aging because the effect is presumably superimposed upon a general inhibition of activity.

In far-red light it is necessary to assume that the excitation of P_{700} is not exchangable with NADP reduction, since the increase in the latter is not accompanied by a decrease in the former. Thus, we are led to the conclusion that perhaps two different sensitizations of the pigment are possible. While the following remarks are highly speculative, our conclusion could fit a recent proposal by Sane and Park [21] who suggested the existence of an isolated

photosystem I in the stroma lamellae and a separate photosystem I associated with photosystem II in the grana.

As far as P_{700} and NADP reduction are concerned, chloroplasts supplied with Mg^{2+} or another cation give similar results as intact algae. In algae, a weak, far-red actinic light provoked an easily measurable P_{700} turn-over with a relaxation time of about 20 msec[3] while the yield of oxygen evolution was low. (Compare this with Table II for chloroplasts.) In contrast, weak short-wavelength light, although presumably giving high yields of oxygen evolution, elicited only a small P_{700} signal. Moreover, partial inhibition of photosynthesis with DCMU resulted in a decrease of oxygen evolution and an increase of relative P_{700} flux (compare Figs 6, 7 and 8). The resemblance between the green alga *Chlorella pyrenoidosa* and chloroplasts appears to be even greater than between the blue-green alga *Anacystis nidulans* and chloroplasts. Thus, by a combination of divalent cations and/or DCMU, we are able to realize any relation of yields between P_{700} and NADP-reduction in chloroplasts and of P_{700} and oxygen evolution in intact cells.

Results obtained with intact cells have, in general, been interpreted as resulting from a very fast reduction of P_{700} under conditions where the quantum yield of photosynthesis is high and a slowed relaxation after inhibition of photosynthesis [22]. Such an interpretation is possible also for chloroplasts in the presence of Mg^{2+}. In this case, the signal due to P_{700} would escape the spectrophotometer whose limit of detection is approximately 0.5 msec. However, we have elsewhere reported kinetic data and results on fluorescence measurements and interpreted these as arguments against the acceleration of P_{700} (ref. 7, 10). In this report we furthermore show that in far-red light, the rate of NADP reduction can increase without any change in the flux through P_{700}. Also, the relaxation time of the pigment remains constant at all DCMU concentrations and after addition of cations.

Still, a recent report by Haehnel *et al.* [18] may be germane to the present discussion. Using a flash apparatus with a high time resolution, the authors detected with benzyl viologen as electron acceptor several P_{700} components relaxing in 20 μsec, 200 μsec, and 20 msec, respectively. Increasing DCMU concentrations progressively inhibited the 200 μsec and increased the 20 msec component. Hence, their data show that a fast component may exist. Although no evidence for or against this interpretation is available, it cannot be excluded that this component participates in the terminal reaction. In this connection, future experiments must establish if results obtained in short flashes of light can be related to our measurements performed in the steady-state. Also, some of the several hypotheses that have been proposed to rationalize the effect of Mg^{2+} in terms of the redistribution of quanta imply that the effect could be related to the intensity of the actinic light [16, 19, 20]. Thus, it further needs to be established whether our results in moderate intensities coincide with measurements of saturation rates [6].

In summary, we feel that the weight of the evidence presented here shows that P_{700} with a relaxation time of 20 msec is not directly involved in the reduction of NADP. Rather, the data show that the two can be antagonistic. If one wishes to maintain the pigment in the well-known series formulation for electron transport, one must assume, against some admittedly indirect evidence [7, 10], that addition of cations accelerates its turn-over so that it escapes detection by the relaxation spectrometer. Conversely, DCMU must slow its turn-over. The question remains, whether the 20 msec component is associated with any terminal reaction. Certainly, in algae it has been possible to correlate P_{700} actively with light-dependent inhibition of respiration and, by inference, with a "cyclic" energy-yielding photosystem (see ref. 23). Perhaps a similar role may be ascribed to it in chloroplasts.

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