THE RELATIONSHIP BETWEEN *P* 700 AND NADP REDUCTION IN CHLOROPLASTS

Hans J. RURAINSKI, Joan RANDLES and George E. HOCH
Department of Biology, University of Rochester, Rochester, New York, 14627, USA

Received 13 November 1970

1. Introduction

Almost all schemes proposed for the electron transport sequence of photosynthesis include *P* 700 as the primary quantum converter of photosystem I. In the currently popular series-scheme [1], this pigment is thought to donate, upon photoexcitation, an electron which eventually reduces nicotinamide adenine dinucleotide phosphate (NADP), a cofactor required for the assimilation of CO_2. The resulting oxidized *P* 700 is thought to be reduced by electrons originating in photosystem II. This scheme predicts that electron flux through *P* 700 will be equal to or greater than the rate of NADP reduction. Attempts to show this relationship yielded exact [2] or approximate [3] correspondence.

We recently reported observations on the relaxation of *P* 700 in intact cells and concluded that the pigment functions in a cyclic photosystem which is only indirectly involved in oxygen evolution and which can be separated from photosynthesis with dichlorophenyl dimethyl urea (DCMU). The results below indicate that *P* 700, instead of participating directly in NADP reduction, competes with the NADP reducing reaction for quanta entering photosystem II and that magnesium ion affects this competition. We shall conclude that *P* 700 and the site of NADP reduction are located in different light reactions and that magnesium ion controls the distribution of excitation to the systems.

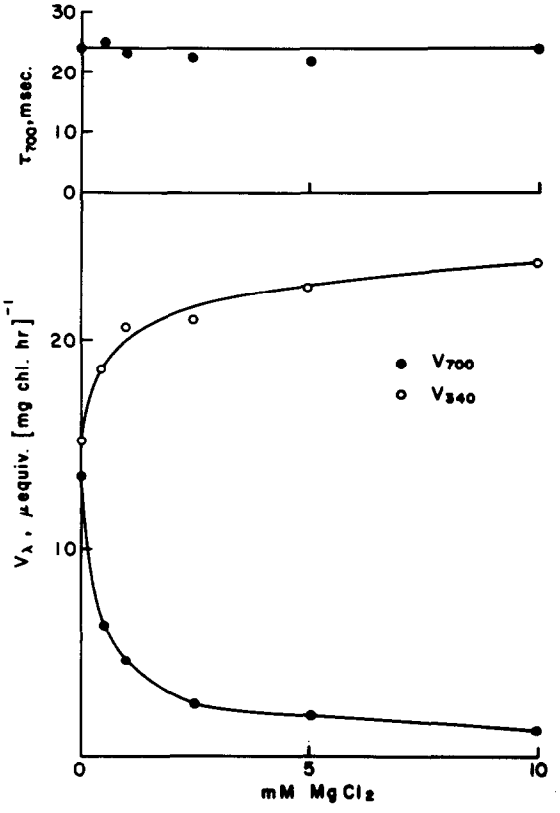

Fig. 1. Changes in reaction rate upon addition of $MgCl_2$. The reaction mixture contained: 50 mM tris (hydroxymethyl) aminomethane pH 7.5, 400 mM sucrose, 20 mM NaCl, 0.25 mM NADP, also per ml: 19 μg chlorophyll, 80 μg ferredoxin, and Mg^{2+} as indicated; total volume 2 ml. Modulation frequency: 39 rads sec^{-1}.

North-Holland Publishing Company – Amsterdam

2. Materials and methods

Chloroplasts were isolated from peas by the method of Nobel [4]; all other experimental conditions have been described previously [3]. Actinic light was filtered through a broad-band interference filter transmitting between 530 and 645 nm. The rate calculations for *P* 700 are based on measurements of absorbance changes and relaxation times observed at 700 nm and are subject to the assumption that *P* 700 is oxidized in light and reduced in darkness in a first-order reaction. To approximate first-order behavior, all measurements were made at low light intensities. The extinction coefficients for the rate calculations were 80 mM^{-1} cm^{-1} for *P* 700 and 6.25 mM^{-1} cm^{-1} for NADP at 340 nm.

3. Results and discussion

Fig. 1 shows that in the absence of magnesium ion, there exists a nearly one-to-one correspondence between electron flux through *P* 700 and the rate of NADP reduction. Upon addition of the ion, however, it becomes apparent that this result is fortuitous. With increasing concentration of Mg^{2+}, the flux through *P* 700 decreases with a concomittant increase in the rate of NADP reduction to the point where the rate of the latter by far exceeds the rate of the former.

Magnesium ion affects the quantum yields of the reactions which are given by the slopes of the lines in fig. 2. The increase in the yield of NADP reduction, which has been previously observed [5], is coupled to a simultaneous decrease in the yield of *P* 700. Again, in the presence of Mg^{2+}, the rate of NADP reduction greatly exceeds that of *P* 700. Fig. 2 also shows that the apparent one-to-one correspondence between the two rates no longer holds in the reaction medium used here even in the absence of Mg^{2+}.

The data are reminiscent of the original observations on *P* 700 in intact cells [6]. Actinic illumination which excited light reaction I elicited good *P* 700 'turn-over' but sub-optimal yields of oxygen evolution. If the exciting light was absorbed by photosystem II, no *P* 700 signal but efficient electron transport was observed. These data and our present observations allow *P* 700 on the path to pyridine nucleotide, as postulated in the series scheme, only if the pigment

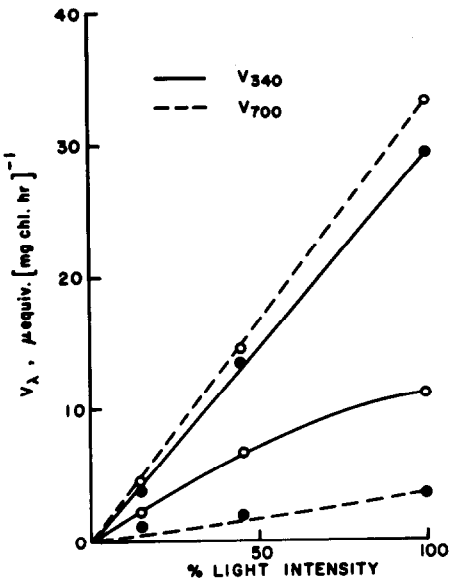

Fig. 2. Changes in quantum yield upon addition of $MgCl_2$. The reaction mixture contained: 15 mM Tricine pH 7.4, 50 mM sucrose, 20 mM NaCl, 0.25 mM KCN, 2.5 mM NH_4Cl, 0.2 mM NADP, also per ml; 16 μg chlorophyll, 50 μg ferredoxin, total volume 2 ml. Modulation frequency: 39 rads sec^{-1}. o: without Mg^{2+}; •: + 5 mM Mg^{2+}.

is reduced so fast by photosystem II that it escapes measurement.

Fig. 1 and table 1 show that the relaxation time, a measure of the time required for the reduction of *P* 700, does not appreciably change upon addition of magnesium ion. Furthermore, an increase in the velocity of *P* 700 to the point where it is undetectable by the relaxation method would not suffice to explain

Table 1
Effects of $MgCl_2$ on relaxation parameters at 700 nm.

	$MgCl_2$ (mM)			
	Fig. 1		Fig. 2	
	0	5	0	5
$\Delta I/I \times 10^5$	24.0	4.6	45.7	4.8
τ (msec)	25	26	20	22
Flux (μequiv [mg chl. hr]$^{-1}$)	14.7	2.8	34.5	3.4

Data taken from figs. 1 and 2.

Table 2
Ratios of rate changes at 700 and 340 nm for two extinction coefficients of *P* 700.

$MgCl_2$ (mM)	Intensity (%)	Decrease in P_{700} flux / Increase in NADP rate	
		80 mM^{-1} cm^{-1}	125 mM^{-1} cm^{-1}
0.5	100	2.0	1.3
1.0	100	1.6	1.0
2.5	100	1.8	1.2
5.0	100	1.6	1.0
5	100	1.7	1.1
5	45	1.8	1.1
5	15	1.7	1.1

Data taken from figs. 1 and 2.

the doubling of the quantum yield of pyridine nucleotide reduction since the relaxation of *P* 700 is fast enough to maintain the observed rates without appreciable loss of quanta*. The decrease in flux is due to a decrease in the amplitude (i.e., concentration) which is consistent with a decreased photon input to the *P* 700 system. We propose, therefore, that *P* 700 and the site of NADP reduction are located in different light reactions and that they compete for quanta of short wavelength. Magnesium ion shifts the advantage to NADP in an as yet unknown mechanism. Additional evidence which supports these conclusions is the observation that Mg^{2+} raises the fluorescence yield of chloroplasts in the presence [7] and absence (Y.S. Li, unpublished results) of DMCU, quite in contrast to the predictions of the series formulation.

The interpretation of the data given above makes the relationship between quanta leaving the *P* 700 light reaction ($\Delta\nu_{700}$) and those arriving in the NADP system ($\Delta\nu_{340}$) important. Attempts to correlate these data are given in table 2. The analysis is handicapped by the uncertainties surrounding the extinction of *P* 700. With the usually assumed value of 80 mM^{-1} cm^{-1} [6], approximately two electrons disappear from the *P* 700 system for each one appearing as NADPH. Recently, however, an extinction coefficient of 125 mM^{-1} cm^{-1} has been reported [8]. Then the ratio is closer to one.

* If *P* 700 were involved in NADP reduction with unit stoichiometry, then $\nu_{340} = [P\,700]_{ox.}\,(1/\tau_{700})$. From the data in fig. 1, ν_{340} = 13.5 μequiv (mg chl. hr)$^{-1}$ and τ_{700} = 0.024 sec; hence $[P\,700]_{ox.} \approx 10^{-4}$ μequiv (mg chl.)$^{-1}$. Assuming one *P* 700/400 chlorophyll molecules, the average concentration of oxidized pigment would be approximately 4% of the total. The increase in quantum yield to be obtained by returning such a small fraction to the reduced *P* 700 pool faster would be negligible.

Acknowledgements

This work was supported by the Public Health Service (GM-13470-03), the Center for Naval Analysis (Sub 5-29101) and by Public Health Service Training Grant (GM-00658-09) from the National Institutes of Health.

References

[1] M. Avron, in: An Introduction to Photobiology, ed. C.P. Swanson (Prentice Hall, Englewood Cliffs, N.J., 1969) p. 144.
[2] B. Kok, in: Photosynthesis Mechanisms of Green Plants, eds. B. Kok and A.T. Jagendorf (N.A.S.–N.R.C. Publication 1145, Washington, D.C., 1963) p. 35.
[3] H.J. Rurainski, J. Randles and G.E. Hoch, Biochim. Biophys. Acta 205 (1970) 254.
[4] P.S. Nobel, Plant Physiol. 42 (1967) 1389.
[5] M. Avron and G. Ben-Hayyim, in: Progress in Photosynthesis Research, ed. H. Metzner (IUBS Publication, Tübingen, 1969), p. 397.
[6] B. Kok and G. Hoch, in: Light and Life, eds. W.D. McElroy and B. Glass (Johns Hopkins Press, Baltimore, 1961) p. 397.
[7] N. Murata, Biochim. Biophys. Acta 189 (1969) 171.
[8] B. Ke, T. Ogawa, T. Hiyama and L.P. Vernon, Biochim. Biophys. Acta, in press.