

QUANTUM REQUIREMENT OF PHOTOSYSTEM I MEDIATED ATP FORMATION IN CHLOROPLAST FRAGMENTS

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Light dependent ATP formation in chloroplasts is coupled to electron transport. The number of quanta required to form a molecule of ATP in various types of electron transport may contribute to our understanding of the nature of coupling of ATP to electron transfer [1]. In contrast to electron transport the light intensity curve for photophosphorylation shows a distinct lag [2, 3]. Large quantum requirements observed in early studies [2] might have been due to lack of appreciation of this low intensity lag. In addition, in ATP formation, which is supported by 'cyclic' electron flow, an initial reduction of the added carrier is required [4] and a proper oxidation-reduction 'poise' should be maintained in order to obtain maximal rates of ATP formation [1]. Sakurai, Nishimura and Takamiya [3] suggested that a pool of high energy intermediates should be filled before photophosphorylation could proceed. Schwartz [5], provided evidence that a build up of proton gradient precedes phosphorylation. He has shown that below a critical actinic light intensity, no ATP synthesis occurs although the proton gradient is formed. Similar observations were made by Dilley [6].

It has been shown that subchloroplast particles (SCP) enriched in System I obtained by the use of digitonin and differential centrifugation support high rates of PMS catalyzed cyclic phosphorylation but their proton pump activity is highly reduced [7]. Recently, we have observed that membranous vesicles derived from the stromal lamellae of spinach chloro-

plasts have similar characteristics [8]. It was, therefore, of interest to study the effect of light intensity in such preparations on ATP synthesis in comparison to chloroplasts. In addition, in studies using digitonin SCP we came to a conclusion that DPIP₂ (in contrast to PMS) supports ATP formation at two sites [9]. If that is the case, quantum requirements for ATP formation, should be lower in the DPIP₂ → MV system than in the PMS system.

In fig. 1 data are presented showing the quantum requirement for ATP formation in chloroplasts and in digitonin SCP with either PMS or DPIP₂. An increase in quantum requirement (i.e. a decrease in efficiency) for ATP formation as the light intensity is decreased, is seen both in chloroplasts and in SCP when PMS is the cofactor. Since in SCP the amount of protons taken up is much smaller than in chloroplasts [7] it is unlikely that the common cause for the increase in quantum requirement at low light intensity is an expression of a lag caused by a critical amount of proton uptake. These experiments have been carried out in the presence of ascorbate since it has been shown that in digitonin-SCP, PMS supported ATP formation depends on an initial reduction of the dye [10]. As can be seen in the data of fig. 1, ATP formation supported by DPIP₂ is more efficient in using the quanta absorbed than the PMS system. Similar data were obtained previously by Avron and Ben-Hayyim [11] for chloroplasts.

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Abbreviations: SCP: subchloroplast particles, MV: methylviologen. DPIP: 2,6-dichlorophenolindophenol. DCMU: 3-(3,4-dichlorophenyl)-1, 1-dimethylurea. PMS: phenazine methosulfate. Chl: chlorophyll.

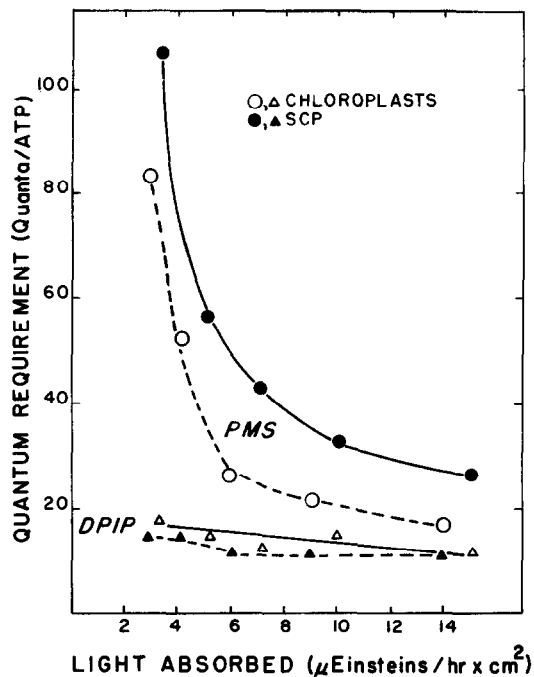


Fig. 1. Quantum requirement of photophosphorylation in chloroplasts and digitonin-derived SCP. Chloroplasts and SCP were prepared as described previously [9]. The reaction mixtures contained (in μ moles): Na Tricine 34 (pH 8.0), NaCl 34, $MgCl_2$ 7, NaPi 7, ADP 2.7, DCMU 0.04 Na-Ascorbate 1.65 and either PMS 0.1, or DPIP 0.55, MV 0.8 and NaN_3 0.8, in a total volume of 2 ml. In addition, each reaction contained ^{32}P ($\sim 10^8$ cpm) and 20 μg chlorophyll. ATP formation was measured as described previously [9]. Reactions were illuminated in a quartz cuvette for 2 min with white light filtered through a Baird-Atomic 710 nm interference filter. Incident light intensity on the cuvette was determined with a Kettering-Yellow Springs Instruments radiometer, Model 65. The percentage of the incident quanta absorbed by the various chloroplast preparations in the sample was determined by measuring the absorption of the sample at 710 nm (corrected for light scattering measured at 750 nm) in a Cary 14 spectrophotometer equipped with a 0–0.1 slide wire and a close-up photomultiplier.

It should be noted that photooxidation of ferrocytochrome C by system I digitonic SCP from spinach was shown to proceed with an efficiency approaching 1 electron/quantum [12].

Quantum requirement studies were also performed with stromal lamellae and grana membranes prepared by French-pressure treatment of spinach chloroplasts [8]. The results are shown in fig. 2. PMS phosphoryl-

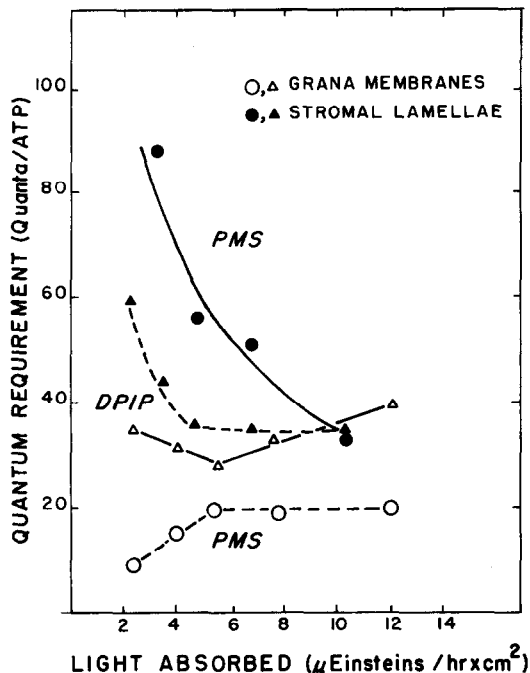


Fig. 2. Quantum requirement for phosphorylation in stromal lamellae and grana membrane preparations derived from whole chloroplasts by French-pressure treatment. Membrane fractions were isolated as described previously [8]. Reaction conditions were as described for fig. 1.

ation is markedly more efficient (lower quantum requirement i.e. higher quantum efficiency) in the stroma particles than in the grana particles. The stromal lamellae preparation is the only preparation in which a decrease in quantum requirement was observed with a decrease in light intensity. Since both the stromal lamellae fraction and the digitonin SCP have a very low proton pump activity [7, 8], it is unlikely that the activity of the proton pump bears a direct relationship to the change in quantum requirement as a function of light intensity. This has also been suggested by Saha, Izawa and Good [13].

It is possible that PMS can donate electrons at several sites in the electron transfer chain, some of which by-pass the phosphorylation site (in analogy with the DPIP $_2$ system [9]). As shown in table 1, the quantum requirement for PMS phosphorylation decreases markedly with decrease in concentration. At higher PMS concentration it seems likely that more electrons are donated to a portion of the electron

Table 1

The effect of PMS concentrations on quantum efficiency of ATP formation in chloroplasts and digitonin-derived SCP.

PMS Concentration (M)	ATP Formed (V) μmoles/mg Chl/hr		I/V (quanta/ATP)	
	Chloroplasts	SCP	Chloroplasts	SCP
1.9×10^{-6}	11.1	8.4	13	17
5.5×10^{-6}	9.6	7.4	15	19
1.7×10^{-5}	6.2	4.9	23	29
5×10^{-5}	0.9	0.5	165	310

Reaction conditions were as described in fig. 1 except that the PMS concentration was adjusted as indicated. Under the conditions of the experiment 145 μ einsteins/hr were absorbed by the preparation.

transport chain which by-passed the ATP forming site. It should be noted that even the highest PMS concentration is below that which has been shown to be inhibitory at high light [14]. We have found no change in the quantum requirement in the system DPIP₂ to MV by changing the DPIP₂ concentration between 0.03–0.3 mM.

In conclusion, it appears that the intensity lag in ATP formation is not directly related to the magnitude of the light dependent proton uptake. It has been shown previously that system I particles, although having a highly reduced proton pump, are able to form ATP catalyzing by system I at high rates at a high light intensity [7]. The present report presents evidence that such preparations are also able to support ATP at low light intensity with the same or even greater efficiency than chloroplasts.

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