EVIDENCE FOR SYSTEM I MEDIATED NON-CYCLIC PHOTOPHOSPHORYLATION IN CHLOROPLASTS

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Introduction

Electron transport from ascorbate + DPIP** to NADP, which is a system I mediated reaction, supports ATP formation [1]. However, ascorbate and DPIP can support ATP formation even in the absence of an electron acceptor [2–4]. The extent of coupling in the system $DPIPH_2$ to NADP as measured by the ratio ATP/e_2 can vary considerably and under some experimental conditions can be lower than 0.2 [4, 5]. On the basis of these and other observations, several workers maintain that ATP formation in the presence of $DPIPH_2$ is of the cyclic type, (both in the presence and absence of an electron acceptor), resembling PMS. According to this formulation, the site of ATP formation is located on the 'cyclic' part of the electron transport chain which connects 'X' with the linear electron transport chain [6]. However, other observations [7–10] indicate that in the system $DPIPH_2$ to NADP (or MV) there is a coupling site on the linear electron transport chain, namely between the site of electron donation and P_{700}.

Since DPIP (in its oxidized form) can be reduced by photosystem I, [11, 12] one can visualize a 'pseudocyclic' system I mediated electron transport from $DPIPH_2$ to the photosystem, to DPIP. The latter might be identical to system I mediated non-cyclic electron flow with regard to the location of the coupling site. It would be difficult to differentiate between a 'pseudocycle' and a genuine 'cycle' of the PMS type. However, if a reductant which can donate electrons to system I, but the oxidized species of which is not reducible by photosystem I, became available, it will be possible to monitor non-cyclic electron flow from donor to acceptor. As shown in the present communication, the compound diaminobenzidine (DAB) meets the above requirements. DAB was shown to be oxidized to a water and lipid insoluble polymer in mitochondria [13] and in chloroplasts [14], as revealed by histochemical studies using the electron microscope. The reaction of electron transport from DAB to MV has been discovered in intact cells and in a chloroplast fragment of *Chlamydomonas reinhardii* by N.H. Chua [15]. In the present work we were able to show unambiguously that system I mediated non-cyclic electron flow supports ATP formation.

2. Materials and methods

Lettuce chloroplasts were isolated and washed as described previously [16]. Chlorophyll determination was performed according to Arnon [17]. Phosphorylation was measured by following ^{32}P incorporation into ATP according to the method of Avron [18]. Other experimental details are specified in the legends of the figures and tables.

3,3'-Diamino benzidine was purchased from Sigma either as the free-base or as the tetrachloride salt.

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** Abbreviations: DPIP, 2,6-dichlorophenolindophenol; MV, methyl viologen; DCMU, 3-(3,4-dichlorophenyl)-1, 1 dimethylurea; PMS, phenazine methosulphate; DAB, 3,3'-diamino benzidine; DTT, dithiothreitol.

3. Results and discussion

Electron transport from water to MV can be monitored by oxygen uptake – in the presence of azide which inhibits endogenous catalase (fig. 1). This reaction is fully inhibited by the addition of 20 μM DCMU. Subsequent addition of 0.3 mM DAB restores oxygen uptake at a rapid rate. In another experiment we have found that addition of 20 μM DCMU after the addition of DAB, has no effect on the rate of oxygen uptake. The electron donor DAB can be oxidized chemically with ferricyanide and re-reduced by DTT, restoring the ability of light dependent electron flow (fig. 1). The addition of DAB and MV in the dark did not support oxygen uptake.

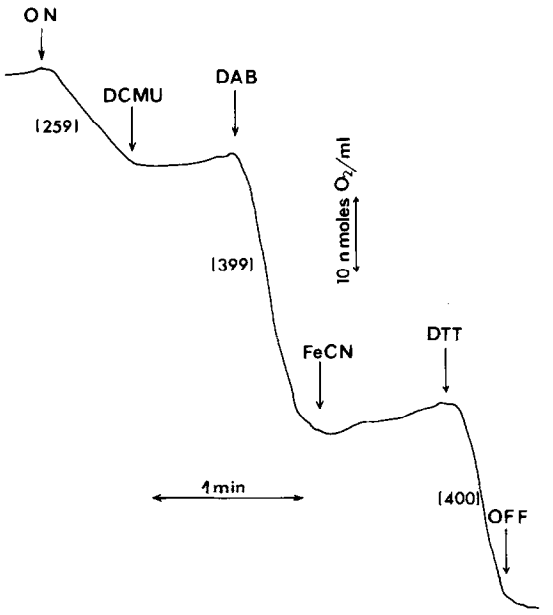

Fig. 1. Electron flow to methyl viologen. The reaction mixture contained the following compounds in a total volume of 3 ml: tricine, 20 mM (pH 8.0); NaCl, 33 mM; MV, 0.2 mM; NaN_3, 1 mM and chloroplasts equivalent to 84 μg chlorophyll. The following compounds were added at the time indicated in the figure: DCMU at 0.02 mM, DAB at 0.33 mM, FeCN at 1.3 mM and DTT at 3.3 mM. Oxygen uptake was measured with a YS1 Clark electrode at 26°C. Illumination was provided by white light filtered through a saturated solution of $CuSO_4$. The light intensity at the level of the vessel was $2.6 \cdot 10^5$ erg·cm^{-2}·sec^{-1}. The numbers in parentheses indicate rates of oxygen uptake expressed as μequivalents of electrons/mg chlorophyll/hr.

Electron transport from DAB to MV is accompanied by ATP formation (table 1). At two alkaline pH values (7.3 and 8.7) the ratio ATP/e_2 is approaching 1.

As shown by several workers [2–4] and discussed in the Introduction, ATP formation in the presence of ascorbate + DPIP does not depend on the addition of a terminal electron acceptor such as MV. This observation is confirmed by the data shown in table 2. However, contrary to the above, when DAB serves as the electron donor, ATP formation (and electron flow which is not shown in this experiment) depends absolutely on the presence of an electron acceptor (table 2). In other experiments we have found that MV can be replaced by substrate amounts of ferredoxin or by catalytic amounts of ferredoxin and NADP. From the results of table 2 it can also be seen that in the system DAB → MV the rate of ATP formation declines with the duration of the reaction, this is due to the decrease in the rate of electron flow. The latter may be caused by the accumulation of oxidized DAB.

The effect of light intensity on ATP formation in the three following systems: PMS, $DPIPH_2$ → MV and DAB → MV, is shown in fig. 2. As can be seen from these data very high light intensities are required to saturate the rate of PMS phosphorylation. This obser-

Table 1
Stoichiometry of ATP Formation to Electron Transport in the system DAB → MV.

pH	Electron transport* (e_2)	ATP**	ATP/e_2
6.5	97	26	0.27
6.9	111	64	0.58
7.3	106	87	0.82
7.8	125	85	0.68
8.2	134	99	0.74
8.7	108	90	0.83

* μmoles 0_2 taken up/mg chlorophyll/hr.
** μmoles formed/mg chlorophyll/hr.
The reaction mixture contained the following in a total volume of 3 ml: tricine-maleate, 13 mM (for each); NaCl, 33 mM; MV, 0.2 mM; NaN_3, 1 mM; DCMU, 10 μM; DAB, 0.14 mM; $MgCl_2$, 1.7 mM; NaP_i, 1.7 mM; ADP, 0.5 mM and chloroplasts equivalent to 140 μg chlorophyll. Illumination was performed by white light which was filtered through a saturated solution of $CuSO_4$. The light intensity at the level of the vessels was 2.6×10^5 erg·cm^{-2}·sec^{-1}. The duration of illumination was 10 sec. Electron flow was assessed by monitoring oxygen uptake.

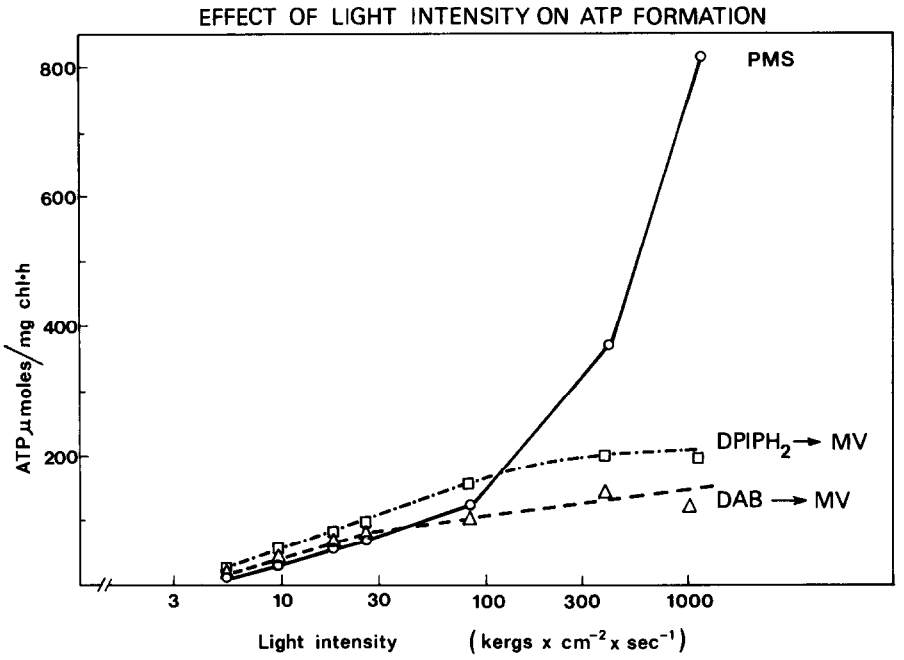

Fig. 2. Effect of light intensity on the rate of ATP formation. The reaction mixture contained the following compounds in a total volume of 3 ml: tricine, 20 mM (pH 7.9); NaCl, 33 mM; DCMU, 10 μM; Na-ascorbate, 1 mM; $MgCl_2$, 3.3 mM; NaP_i (containing approximately 10^6 cpm of ^{32}P) at 3.3 mM and ADP at 1 mM. It also contained PMS 30 μM or DPIP, 0.1 mM or DAB, 0.33 mM. In addition it contained chloroplasts equivalent to 60 μg chlorophyll. Illumination was provided by white light at the indicated intensity. The duration of the reaction was 10 sec.

Table 2
The effect of MV on the rate of ATP formation.

Duration of illumination (sec)	DAB → MV (μmoles/ATP/mg chlorophyll/hr)		$DPIPH_2$ → MV	
		−MV		−MV
5	116	0	122	129
10	108	0	113	114
20	90	2	100	106
30	68	3	98	100
60	40	4	87	87

The reaction mixture contained the following in a total volume of 3 ml: tricine, 20 mM (pH 8.0); NaCl, 33 mM; DCMU, 10 μM; NaP_i, 3.3 mM (containing approximately 10^6 cpm of ^{32}P); $MgCl_2$, 3.3 mM; ADP, 1 mM; Na-ascorbate, 1 mM and either DAB, 0.33 mM or DPIP, 0.1 mM. It also contained chloroplasts equivalent to 60 μg chlorophyll. MV when present was added at a concentration of 0.2 mM. Otherwise experimental conditions as in table 1.

vation confirms a previous report [18]. The two other reactions, on the other hand, are saturated at a light intensity of approximately $3 \cdot 10^5$ erg·cm^{-2}·sec^{-1} (fig. 2). Since the system DAB → MV was shown in the present work to be of the non-cyclic type it can be concluded that the site of ATP formation is located between the site of electron donation by DAB and P_{700}. By analogy we suggest that the site of ATP formation catalyzed by $DPIPH_2$ is between the site of electron donation by $DPIPH_2$ and P_{700}. The site of ATP formation in the latter should not be dependent on whether 'XH' is oxidized by DPIP or by MV (i.e. whether electron transport is of the non-cyclic type or the pseudocyclic type).

4. Conclusions

The compound DAB can serve as an electron donor to photosystem I in isolated chloroplasts in the presence of DCMU. Since the oxidized moiety of DAB is not reducible by photosystem I, electron transport with this donor depends absolutely on the presence of a terminal electron acceptor. Electron transport

from DAB to MV supports ATP formation with a stoichiometry of ATP/e_2 which is approaching 1. The dependence of ATP formation on light intensity in the system $DPIPH_2$ → MV is similar to that of the system DAB → MV and differs markedly from the PMS system indicating the similarity between the DAB and $PDIPH_2$ systems.

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