On the site of ATP formation in photophosphorylation*

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The photoreduction of TPN by isolated chloroplasts can be performed with either water or ascorbate + 2,6-dichlorophenol indophenol (DPIP) as the electron donor (1). Vernon and Zaugg demonstrated that while the first reaction was very sensitive to inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the latter was relatively resistant (1). It was suggested that when ascorbate + DPIP donate the electrons a shorter fragment of the electron transport chain is utilized than when water is the electron donor. This interpretation was further supported by several other findings (2,3).

When ATP production was shown to be coupled to the reduction of TPN with water as the electron donor (4) it became evident that one could localize the site of ATP formation as being before or after the point of entry of the electrons supplied by the ascorbate + DPIP couple. This could be achieved by determining whether or not ATP production was coupled to the reduction of TPN by the ascorbate + DPIP couple. Indeed, Losada et al. (5) have shown that when such measurements were made about 1 molecule of ATP was formed for each molecule of TPN reduced. It was concluded therefore that the site of ATP formation in photophosphorylation was between the point of entry of electrons from the ascorbate + DPIP couple and TPN.

The finding that reduced DPIP can by itself catalyse cyclic photophosphorylation (6,7,8) raised our doubts as to whether the phosphorylation which accompanied the photoreduction of TPN by ascorbate + DPIP was related to the electron transfer process leading to TPN reduction (9). These doubts were supported by our previous observation that the reduction of TPN by ascorbate in the absence of DPIP was not associated with any significant phosphorylation (10).

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Table 1 illustrates that all the phosphorylation associated with TPN reduction by the ascorbate + DPIP couple could be accounted for by the cyclic phosphorylation catalysed by ascorbate + DPIP. It further illustrates that the optimal concentration of reduced DPIP for catalysing phosphorylation is higher (9), than the optimum for its acting as an electron donor for TPN reduction. It could also be shown that by varying the amount of ferredoxin added to the reaction mixture the ratio of the number of molecules of ATP formed to those of TPN reduced ($P/_{2e}$-ratio) obtained could be varied from 0.2 to infinite.

TABLE I

The site of ATP formation during the photoreduction of DPIP

Additions	ATP formed +TPN	ATP formed -TPN	TPN reduced
	μmoles per mg chlorophyll per hour		
None	180	6	169
Ascorbate	206	-	163
Ascorbate + DPIP (0.2)	206	-	133
DCMU	4	8	7
DCMU + ascorbate	6	11	20
DCMU + ascorbate + DPIP (0.05)	16	18	37
DCMU + ascorbate + DPIP (0.2)	40	42	71
DCMU + ascorbate + DPIP (1.50)	80	82	61

The experiments were performed with once washed chloroplasts prepared as previously described (11), but washed in a medium lacking ascorbate. The reaction mixture contained in μmoles: Tris-HCl, pH 7.8- 45; NaCl - 60; $MgCl_2$ - 12; Na, K phosphate, pH 7.8 - 12; (containing 2 x 10^6 cpm of P^{32}), ADP - 4; TPN - 1; chloroplasts containing 40 μg of chlorophyll, a saturating amount of ferredoxin prepared as described by Hill and Bendall (12) as far as and including the column fractionation on DEAE-cellulose, and water to a total volume of 3.0 ml. DCMU and ascorbate, where indicated, were added at 2 x 10^{-7} M and 6.7 x 10^{-3} M, respectively. The amount of DPIP added in μmoles per 3 ml is indicated in brackets. Reaction time - 3 min. Light intensity - 40,000 lux.

We believe that the evidence presented strongly supports the hypothesis that the site of ATP formation during the photoreduction of TPN must lie between water as the electron donor and the point of entry of electrons from the ascorbate + DPIP couple.

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Note: Since submission of the manuscript, two recent publications came to the attention of the author which arrive at the same conclusions using different criteria:

Wessels, J.S.C., Biochim. Biophys. Acta 79, 640 (1964).

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