PHOSPHORYLATION COUPLED TO NON-CYCLIC ELECTRON FLOW IN PHOTOSYSTEM I

N. SHAVIT and V. SHOSHAN

Division of Life Sciences, Negev Institute for Arid Zone Research

and

Biology Department, University of the Negev, Beer-Sheva, Israel

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1. Introduction

Electron flow pathways in islated chloroplasts are associated with one or with both photosystems, and may be of a cyclic or a non-cyclic nature. Photoinduction of such electron flow paths is accompanied by the synthesis of ATP [1]. The localization of the sites of ATP formation along these electron flow pathways is a problem of great interest. A site of phosphorylation prior to the reduction of cytochrome *f* has been established. It was suggested that this site is functional in the electron flow from water to ferricyanide or to $NADP^+$ [2]. In contrast, controversy exists as to the phosphorylation accompanying the electron flow from ascorbate-DCIP to $NADP^+$, in the presence of DCMU. This arises from a simultaneous cyclic phosphorylation reaction, which accompanies the noncyclic electron flow [3–5].

It was recently shown that in chloroplasts ATP inhibited both phosphorylation and the coupled electron flow [6]. Furthermore, this inhibition of electron flow was released in the presence of an uncoupler. Thus this specific effect of ATP on coupled electron flow can be used to determine whether the electron flow from ascorbate-DCIP to $NADP^+$ is indeed coupled to phosphorylation. The present data support the concept of a phosphorylation site in the electron flow path from ascorbate to $NADP^+$. In view of the evidence available that cytochrome *f* does not participate in this electron flow [2, 7], it is concluded that two phosphorylation sites may operate in the electron flow from water to $NADP^+$.

2. Materials and methods

Chloroplasts were isolated from fresh market lettuce leaves by standard procedures [6]. $NADP^+$ reduction was followed either directly at 340 nm with a Cary spectrophotometer, model 15, adapted for illumination, or determined fluorometrically as described [8]. ATP formation was assayed as described [6]. Ferredoxin used in these experiments was generously provided by Dr. M. Avron, Weizmann Institute of Science, Rehovot, Israel.

3. Results

The inhibition of $NADP^+$ reduction from water by ATP is given in fig. 1A. The basal reduction rate, i.e., in the absence of phosphorylating agents, was only slightly inhibited. This situation is similar to that described earlier for ferricyanide reduction [6]. As can be seen from fig. 1B the basal rate of $NADP^+$ reduction from ascorbate-DCIP, was stimulated by phosphorylating agents. This basal rate was again not affected by ATP. However the coupled rate of reduction, in the presence of phosphorylating agents, was inhibited by ATP in a manner quite analogous to that obtained when the electron donor was water.

Abbreviations:

DCIP:	2, 6 dichlorophenol indophenol
DCMU:	3-(3, 4-dichlorophenyl)-1,1-dimethyl urea
CHL:	Chlorophyll
P/e_2:	ATP/NADPH formed

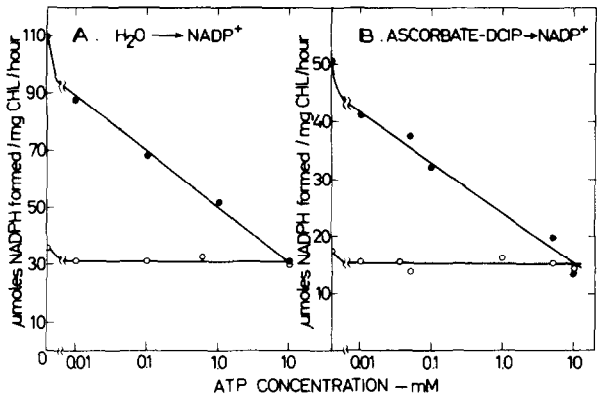

Fig. 1. (A) The effect of ATP on the rate of reduction of $NADP^+$ from water. Reaction mixtures contained the following components in μmoles in a total volume of 3 ml at pH 7.8: tris-HCl, 30; KCl, 60; $NADP^+$, 1; a saturating amount of ferredoxin and chloroplasts containing 64 μg chlorophyll. $NADP^+$ reduction was followed spectrophotometrically at 340 nm as described in Methods. Red light was provided by illumination with a 150-W quartz iodine lamp, through a heat filter and a Corning filter 2304. Light intensity was 1.8×10^5 erg cm^{-2} sec^{-1}. A Kodak wratten filter 39 and a 1 cm. of saturated $CuSO_4$ solution were used as filters before the photomultiplier. ADP, 1; Pi, 2 and $MgCl_2$, 5 were added as indicated. (B) The effect of ATP on the rate of reduction of $NADP^+$ from ascorbate-DCIP. Reaction mixtures and other conditions as in fig. 1 (A), but with ascorbate, 20 μmoles; DCIP 0.2 μmoles and DCMU, 1.8 nmole. • reduction (+ADP, Pi and $MgCl_2$); ○ reduction (−ADP, −Pi, $-MgCl_2$).

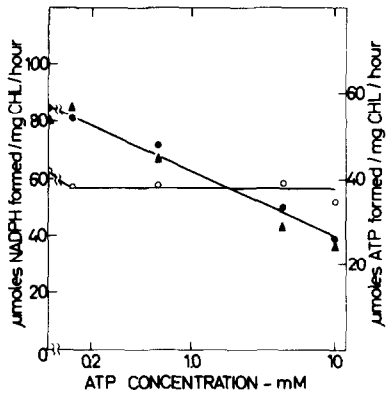

Fig. 2. The effect of ATP on the rate of phosphorylation and electron flow from ascorbate-DCIP to $NADP^+$. • reduction (+ ADP); ○ reduction (−ADP); ▲ ATP formation. Reaction mixtures as in 1B, except that the basal electron flow was determined in the absence of only ADP; chloroplasts containing 66 μg chlorophyll were added; under phosphorylating conditions ^{32}P containing 6×10^5 cpm per flask was added. Light intensity was 160,000 Lux of white light. NADPH was assayed fluorimetrically as described in Methods.

The rate of ATP synthesis was inhibited by ATP to a similar degree as was that of $NADP^+$ reduction from ascorbate-DCIP (fig. 2). The P/e_2 ratio was not changed. Values of 0.65 without ATP and 0.62 with 10 mM ATP were obtained. The rate of $NADP^+$ reduction without ADP was stimulated by its addition. It was reported that varying the concentration of DCIP, in this system, affects more the phosphorylation than the electron flow rate [3–5]. Varying the concentration of DCIP did not, however, affect the degree of inhibition of $NADP^+$ reduction by ATP (table 1). The rate of $NADP^+$ reduction from ascorbate-DCIP was stimulated by nigericin and this uncoupled rate was not affected by ATP.

Table 1
The effect of varying DCIP concentration and an uncoupler on the inhibition of $NADP^+$ reduction by ATP.

Additions	Ascorbate-DCIP → $NADP^+$ (μmoles NADPH formed/mg CHL/hour)	Inhibition (%)
DCIP (0.02)	43.6	
DCIP (0.02) + ATP	29.2	33
DCIP (0.1)	75.6	
DCIP (0.1) + ATP	51.7	32
DCIP (0.2)	69.3	
DCIP (0.2) + ATP	42.8	38
DCIP (0.2) + nigericin	139.3	
DCIP (0.2) + nigericin + ATP	140.8	0

Reaction mixtures and assay conditions as described in fig. 1B. Chloroplasts containing 74 μg chlorophyll were added. The concentration of ATP was 1 mM and that of nigericin 0.5 μM. The numbers in brackets represent μmoles of DCIP added.

4. Discussion

The difficulty in assessing whether the electron flow from ascorbate to $NADP^+$ is accompanied by a coupled phosphorylation, lies in the fact that reduced DCIP, can give rise to a simultaneous cyclic phosphorylation. Thus phosphorylation could occur at a site which is not intimately linked to the electron flow from ascorbate through photosystem I to $NADP^+$. The mechanism of ATP interaction with the electron transport chain is not yet clear. However, the fact that ATP inhibits the coupled electron flow rate only, suggests an interaction with some intermediate stages in the photophosporylation reaction. This is also supported by the effect of ATP on the two stage phosphorylation reaction. In this reaction ATP had an inhibitory effect only during the dark stage*. ATP also inhibited the light induced proton gradient* measured in the presence of phosphorylating agents, by the distribution of a labelled amine derivative [9]. Thus, we can envisage an interaction between ATP and electron flow only when electron flow is coupled to phosphorylation, probably through an intermediate step(s). The inhibition by ATP of a simultaneously occurring cyclic electron flow should not be expected to influence a priori a non-cyclic electron flow from ascorbate to $NADP^+$, which is not coupled to phosphorylation.

Several authors have suggested two phosphorylation sites associated with the electron flow from water to $NADP^+$ based on either the effects of electron donating compounds [10] or on differential inhibition of phosphorylation [11, 12]. One site of phosphorylation was postulated to be closely associated with water oxidation [10]. Other authors proposed that the cyclic phosphorylation site is located on a part of the cyclic electron flow chain not common with the chain of the non-cyclic reaction from ascorbate- DCIP to $NADP^+$ [13, 14]. The latter concluded that no site of phosphorylation exists in this non-cyclic electron flow pathway. Accepting the evidence that cytochrome *f* does not participate in the $NADP^+$ reduction from ascorbate-DCIP [2, 7] and that a phosphorylation site precedes the reduction of this cytochrome [2] it is reasonable to conclude from the present observations that a site of phosphorylation occurs in the non-cyclic electron flow from ascorbate-DCIP to $NADP^+$. Thus, two phosphorylation site may operate in the electron flow from water to $NADP^+$.

As was described, ATP acted as an energy transfer inhibitor, since it affected the coupled electron flow only and the inhibition was released by an uncoupler. However, other known energy transfer inhibitors were reported not to inhibit the reduction of $NADP^+$ from ascorbate-DCIP, but rather to stimulate it [15]. This discrepancy remains unexplained. Although the effect of ATP appears to be similar to that of an energy transfer inhibitor, its mode of action could differ from that of phlorizin. ATP could exert its effect on electron flow and phosphorylation by affecting the enzyme(s) participating in the terminal step(s) of the phosphorylation reaction. The inhibitory effect could also be the result of a change in the internal pH or on the transport of a newly synthesized ATP away from its site of formation. Such possibilities are currently being investigated.

* A. Herscovici, V. Shoshan and N. Shavit, unpublished results.

References

[1] M. Avron, in: Current topics in Bioenergetics, ed. D.R. Sanadi, Vol. II, (Academic Press, New York, 1967) p.1.

[2] M. Avron and B. Chance, in: Currents in Photosynthesis, eds. J.B. Thomas and J.C. Goedheer (Ad. Donker, Rotterdam, 1966) p. 455.

[3] J.S.C. Wessels, Biochim. Biophys. Acta 72 (1964) 640.

[4] M. Avron, Biochem. Biophys. Res. Commun. 17 (1964) 430.

[5] Z. Gromet-Elhanan, Biochim. Biophys. Acta 131 (1967) 526.

[6] N. Shavit and A.Herscovici, FEBS Letters 11 (1970) 125.

[7] D.S. Gorman and R.P. Levine, Proc. Natl. Acad. Sci. U.S. 54 (1965) 1665.

[8] G. Ben-Hayyim, Z. Gromet-Elhanan and M. Avron, Anal. Biochem. 28 (1969) 6.

[9] H. Rottenberg, T. Grunwald and M. Avron, FEBS Letters 13 (1971) 41.

[10] H. Böhme and A. Trebst, Biochim. Biophys. Acta 180 (1969) 137.

[11] M. Avron and N. Shavit, Biochim. Biophys. Acta 109 (1965) 317.

[12] C.C. Black, Biochem. Biophys. Res. Commun. 28 (1967) 985.

[13] G.A. Hauska, R.E. McCarty and E. Racker, Biochim. Biophys. Acta 197 (1970) 206.

[14] Z. Gromet-Elhanan and N. Redlich, European J. Biochem. 17 (1970) 523.

[15] Z. Gromet-Elhanan, Arch. Biochem. Biophys. 123 (1968) 447.