THE INHIBITION OF PHOTOREACTIONS OF CHLOROPLASTS BY IOXYNIL

Zippora Gromet-Elhanan
Biochemistry Section, Weizmann Institute of Science
Rehovoth, Israel

Received November 27, 1967

Controversial results have recently appeared in the literature with regard to the effect of ioxynil (4-hydroxy-3,5-diiodo benzonitrile) on photoreactions of chloroplasts. Paton and Smith (1965a) observed ioxynil to inhibit noncyclic phosphorylation with NADP as well as the cyclic one with PMS* - all at the same range of concentration. Also, by following the photoreduction of plastoquinone they found that ioxynil, unlike O-phenanthroline and DCMU, inhibited the electron transport when either water or ascorbate-DCIP served as the electron donor (Paton and Smith, 1965b). On the other hand, Friend and Olsson (1967) reported that ioxynil acted exactly like O-phenanthroline and DCMU, inhibiting the photoreduction of plastoquinone and NADP only when water served as the electron donor. In this context it is also of interest that BDHB, which is structurally quite similar to ioxynil, was found to act like DCMU, since it inhibited the PMS-catalyzed cycling photophosphorylation much less than noncyclic photophosphorylation (Avron and Shavit, 1965).

When the effect of ioxynil on various photoreactions of chloroplasts was reinvestigated, it was found to inhibit completely all photoreactions with water as the electron donor at concentrations below 10^{-6} M. It also inhibited cyclic photophosphorylation but only at a 200-1000-fold higher concentration, while the photoreduction of NADP with ascorbate-DCIP was resistant even to 10^{-3} M ioxynil.

METHODS - Chloroplasts were prepared from lettuce (Lactuca sativa var.romaine) leaves as described by Avron (1961a) but washed once in a medium without ascorbate. Reactions were run in test-tubes inside a water bath maintained at 20°, and illuminated for 3 min. at 100,000 lux. The reaction mixtures contained the following components

*The abbreviations used are: PMS, phenazine methosulphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenol indophenol, BDHB, n-butyl-3,5-diiodo-4-hydroxylbenzoate.

in μmoles in a total volume of 3 ml ; Tris-HCl (pH 7.8), 80; NaCl, 40; $MgCl_2$, 8; ADP, 5; Na, K phosphate, 10 (containing about 4×10^6 cpm of P^{32}) and once -washed chloroplasts containing 30-60 μg chlorophyll. In addition, the reactions contained 1.5 μmoles of ferricyanide or 1.0 μmole of NADP and saturating amounts of ferredoxin, or NADP and ferredoxin together with 0.2 μmole DCIP, 20 μmoles ascorbate and 2×10^{-6}M DCMU. The PMS series contained 0.1 μmole of PMS, and when indicated 20 μmoles of ascorbate.

Reduction of ferricyanide was assayed according to Avron and Shavit (1963). NADP reduction was measured by the method of Ben Hayyim et al. (1967) and ATP formation was determined as described by Avron (1960) using the modifications outlined by Gromet-Elhanan (1967b).

RESULTS AND DISCUSSION - Ioxynil was found to be a very potent inhibitor of photoreduction and photophosphorylation when water was the electron donor (Figure 1). Like the alkyl-hydroxy-quinoline N-oxides (Avron, 1961b) it was more effective with ferricyanide than with NADP as the electron acceptor, giving 50% inhibition at 7×10^{-8}M and 5×10^{-7}M respectively. With ascorbate-DCIP as an electron donor ioxynil at these low concentrations affected neither the phosphorylation nor NADP reduction, as is the case with many other inhibitors which supposedly inhibit close to the oxygen evolution site (Avron and Shavit, 1965).

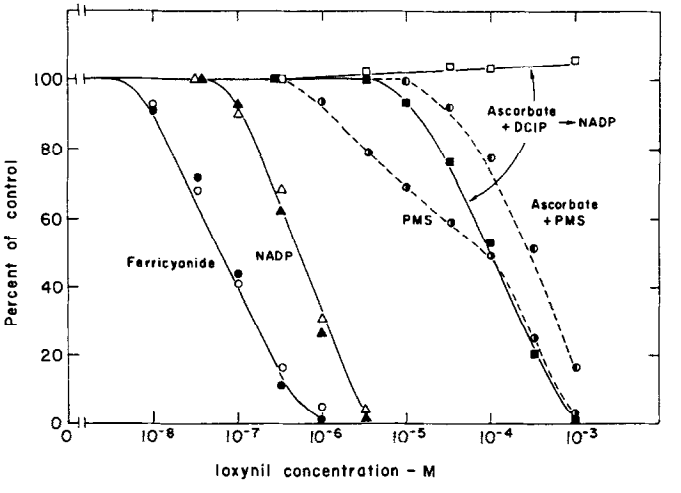

Figure 1 - Effect of Increasing Concentrations of Ioxynil on Chloroplast Photoreactions. Open symbols represent photoreduction. Semiclosed and closed symbols represent photophosphorylation. Control activity (expressed in μmoles/mg chlorophyll/hr) corresponds to the following values : 0, ● with ferricyanide 277 and 133; Δ,▲ with NADP 69 and 82; □ , ■ with ascorbate-DCIP to NADP 41 and 64 respectively. Control activity with PMS was 417 and with ascorbate-PMS 504.

The phosphorylation accompanying the electron flow from ascrobate-DCIP to NADP was inhibited when the concentration of ioxynil was raised, giving 50% inhibition at 10^{-4}M. However, the photoreduction of NADP in this system was resistant to ioxynil even at concentrations which totally blocked all types of photophosphorylation (Figure 1).

In contrast with the results of Paton and Smith (1965a) the PMS-catalyzed cyclic phosphorylation was not inhibited by ioxynil at the concentrations required to inhibit noncyclic photophosphorylation, but rather followed the pattern of inhibition of the phosphorylation with ascorbate-DCIP as the electron donor (Fig. 1). The omission of ascorbate increased somewhat the sensitivity of the PMS system to ioxynil. A similar protective effect of ascorbate was observed when the PMS-catalyzed phosphorylation was inhibited by DCMU (Jagendorf and Margulies, 1960; Wessels, 1962).

In the last few years many compounds were reported to exert effects similar to those reported here with ioxynil, namely, inhibition of electron transport and photophosphorylation with water as the electron donor as well as cyclic photophosphorylation, without affecting the electron transport from ascorbate-DCIP to NADP. These include salycilaldoxime (Trebst, 1963); various 2-trifluoromethyl benzimidazoles (Buchel et al., 1966; Gromet-Elhanan, 1967a,b); Dio-9 and phlorizin (Gromet-Elhanan, 1967a,b) and phenol (Neumann and Drechsler, 1967). However, unlike ioxynil, all these compounds blocked noncyclic and cyclic photophosphorylation at the same range of concentrations.

The effects of the above mentioned compounds, as well as ioxynil, can be explained by proposing that they act in the same manner at two different sites : one near photoreaction II and the second near photoreaction I. The resistance of the photoreduction of NADP with ascorbate-DCIP as the electron donor to all these compounds implies that the second site of inhibition cannot be located on the electron transport chain between ascorbate-DCIP and NADP, but is rather located on that part of the electron transport chain which is exclusive for the cyclic system. The absence of phosphorylation in the resistant system would indicate that there is no site of phosphorylation on the electron transport chain leading from ascorbate-DCIP to NADP.

ACKNOWLEDGEMENTS

I would like to thank Dr. B. J. Heywood of May and Baker Ltd., Dagenham, Essex for a gift of ioxynil. I wish to thank Miss O. Cohen for technical assistance.

REFERENCES

Avron, M. (1960). Biochem. Biophys. Acta 40, 257.
Avron, M. (1961a). Anal. Biochem. 2, 535.
Avron, M. (1961b). Biochem. J. 78, 735.
Avron, M. and Shavit, N. (1963). Anal. Biochem. 6, 549.
Avron, M. and Shavit, N. (1965). Biochim. Biophys. Acta 109, 317.
Ben Hayyim, G., Gromet-Elhanan, Z. and Avron, M., in preparation.
Buchel, K. H., Draber, W., Trebst, A. and Pistorius, E. (1966). Zeit. Naturforsch. 21b, 243.
Friend, J. and Olsson, R. (1967). Nature 214, 942.
Gromet-Elhanan, Z. (1967a). Abstracts, 7th International Congress of Biochemistry, Tokyo, H-43.
Gromet-Elhanan, Z. (1967b). Arch. Biochem. Biophys. in press.
Jagendorf, A. T. and Margulies, M. (1960). Arch. Biochem. Biophys. 90, 184.
Neumann, J. and Drechsler, Z. (1967). Plant Physiol. 42, 573.
Paton, D. and Smith, J. E. (1965a). Weed Res. 5, 75.
Paton, D. and Smith, J. E. (1965b). Nature 207, 1211.
Trebst, A. (1963). Zeit. Naturforsch. 18b, 817.
Wessels, J. S. C. (1962). Biochim. Biophys. Acta 65, 561.