Inhibitors of Photosynthetic Electron Transport. The Properties of Diazidodialkylbenzoquinones

Hagan Bayley

Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, USA

Z. Naturforsch. **34 c**, 490–492 (1979);
received February 6, 1979

Photosynthetic Electron Transport, Azidoquinones, Photoaffinity Labels

The potential photoaffinity reagents 2,5-diazido-3-methyl-6-isopropyl-1,4-benzoquinone (DAZMIB) and 2,5-diazido-3,6-dimethyl-1,4-benzoquinone (DAZDMQ), which are analogs of the well-known inhibitor 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB), block photosynthetic electron transport in spinach chloroplasts. On irradiation, at relatively high concentrations, DAZMIB inhibits electron flow irreversibly. However, when [^{3}H]DAZDMQ was photolyzed with chloroplasts, no labeling of the membrane proteins was observed. Rearrangements of the nitrenes derived from the oxidized or reduced azidoquinones that might account for this behaviour are discussed.

Introduction

Trebst *et al.* [1] discovered that 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB) is a powerful inhibitor of photosynthesis, blocking electron transport in chloroplasts between plastoquinone and plastocyanin. The precise mode of action of DBMIB is unknown. We have investigated analogous diazidoquinones as photoaffinity reagents for the supposed binding site of the inhibitor [2]. These reagents proved to be unsuited to their purpose. But, because of recent interest in such compounds [3, 4] we present details of our findings here.

Materials and Methods

DBMIB was prepared by the method of Trebst *et al.* [1]. 2,5-Diazido-3-methyl-6-isopropyl-1,4-benzoquinone (DAZMIB) was prepared by treating DBMIB with NaN_3 in methanol for 3 h at room temperature (*cf.* [5]). 2,5-Dibromo-3,6-dimethyl-1,4-benzoquinone (DBDMQ) and the corresponding diazidoquinone (DAZDMQ) were prepared according to Smith and Nichols [6] and Zee-Cheng and Cheng [7] respectively. [^{3}H]DAZDMQ was prepared in eight steps from 2,5-dimethylphenol (details are available on request). The final product had a specific radioactivity of 45 mCi mmol^{-1} and was both chemically and radiochemically pure. 2,5-Diazido-3,6-dimethyl-1,4-benzohydroquinone and 2-amino-5-azido-3,6-dimethyl-1,4-benzoquinone were prepared by modifications of the methods of Moore and Shelden [5]. The hydroquinone was stored as a dilute $CHCl_3$ solution at 4 °C under N_2 and used within 12 h.

Spinach chloroplasts were isolated by a modification of the method of Avron [8] (J. Li, personal communication) and were used at once. $NADP^+$ reduction was measured by the method of Levine and Smillie [9]. The reaction mixture (1.0 ml) contained ferredoxin (2.8 nmol), NADP-reductase (0.05 units), chloroplasts (15 – 20 μg of chlorophyll) and $NADP^+$ (0.5 μmol) in 40 mM Tricine, pH 7.8, containing KCl (20 mM), $MgCl_2$ (2.5 mM) and methylamine (3 mM). Ferredoxin, reductase and $NADP^+$ were omitted from the reference cell. Quinones were added to both cells in methanol (final concentration 0.2 – 1.0%).

Results and Discussion

DAZMIB proved to be a powerful inhibitor of electron transport, but at low concentrations the inhibition was largely relieved by irradiation during the assay. This is clear in Fig. 1 where 1.25 μM DAZMIB, sufficient to give an initial inhibition of 90%, was used. A similar result was recently reported by Oettmeier *et al.* [4]. At higher concentrations of DAZMIB partial irreversible inhibition of electron transport occurred (Fig. 2). The disappearance

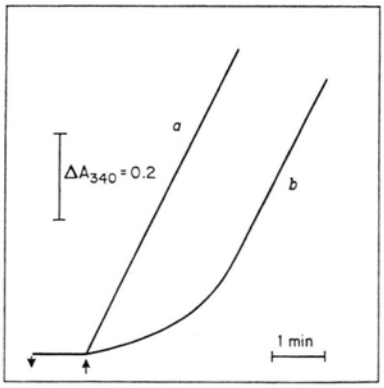


Fig. 1. Assays of photosynthetic electron transport ($NADP^+$ reduction). a. No addition. b. In the presence of 1.25 μM DAZMIB. ↓ actinic light off, ↑ actinic light on.

Reprint requests to Dr. H. Bayley.

0341-0382 / 79 / 0500-0490 $ 01.00/0

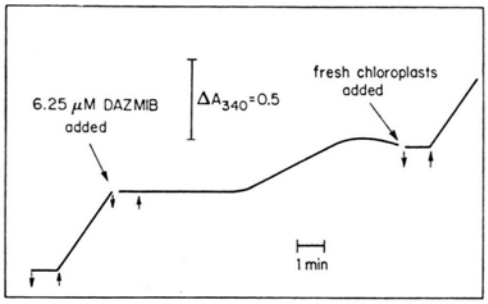
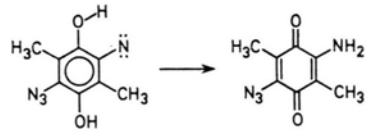


Fig. 2. Assay of photosynthetic electron transport with the addition of 6.25 μM DAZMIB. See Results and Discussion for details and Fig 1. for symbols.

of the azide monitored by difference spectroscopy corresponded to the partial recovery of the chloroplasts. Both the rate of $NADP^+$ reduction after photolysis of the azide was complete and the final ΔA_{340} were greatly reduced. The nature of the inhibition was rather complex. Photolysis of the chloroplasts in the absence of inhibitor had no effect, neither did the addition of DAZMIB which had been prephotolyzed in buffer. However, DAZMIB prephotolyzed with all the assay ingredients except chloroplasts (*i. e.*, including ferredoxin, reductase and $NADP^+$) did inhibit electron transport. Chloroplasts in buffer alone were inhibited after irradiation with DAZMIB but the presence of ferredoxin, etc., enhanced their inhibition. When fresh chloroplasts were added to an assay mixture containing chloroplasts inhibited in any of the above ways, the full rate of electron transport was regained. Therefore none of the mixtures contained free, photogenerated inhibitors.

The inhibition of a biological system by a photoaffinity reagent cannot be interpreted with certainty to signify covalent reaction of the reagent at its site of action [10]. We therefore decided to use a radiolabeled reagent to try and ascertain if any specific photolabeling was occurring. [^{3}H]DAZDMQ was more readily accessible than [^{3}H]DAZMIB. Both DAZDMQ and DBDMQ were approximately ten times less active than DAZMIB (pI_{50} * = 5.7 for both compounds). [Oettmeier *et al.* [4] have determined the pI_{50} of DBDMQ to be 5.9 in a slightly different assay.] To our disappointment when membranes, irradiated with 10 μM [^{3}H]DAZDMQ, were recovered by centrifugation, lyophilized and extracted with ether, only ~0.5% of the initial radioactivity was recovered in the pellet. The amount of label remaining in the unphotolyzed control, after similar treatment, was approximately the same. The "labeled" membranes were subjected to sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. No labeled protein bands were revealed by fluorography.

Even in the absence of specific labeling, a high degree of nonspecific labeling would be expected from a lipophilic aryl azide [11]. However, azidoquinones are known to rearrange on photolysis to 2-cyanocyclopent-4-ene-1,3-diones [12]. But since the process of photoaffinity labeling may resemble solid state or intramolecular photochemistry [13], it was not obvious that this reaction would occur at a tight binding site where the immobilized intermediate, a nitrene or aziridine, might be trapped by a nucleophile. Moreover, it is not known whether the active form of the inhibitor is the oxidized or reduced quinone. Oettmeier *et al.* [4] have shown that reduced DBMIB has the same pI_{50} as oxidized DBMIB. Here though, the possibility exists that the intermediate might undergo an internal disproportionation:



We have obtained spectrophotometric evidence for this reaction. Reduced DAZDMQ (λ_{max} 272, 310 nm) was irradiated in methanol. A_{330} characteristic of the aminoazidoquinone (λ_{max} 327, 510 to 530 nm) was plotted against the decrease in A_{270} (representing the destruction of the hydroquinone) (Fig. 3). An intermediate with the absorption properties of the aminoazidoquinone is formed. After

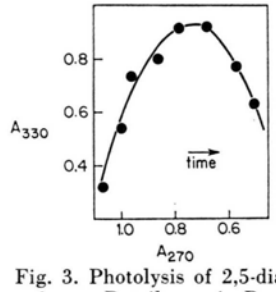


Fig. 3. Photolysis of 2,5-diazido-3,6-dimethyl-1,4-benzohydroquinone. Details are in Results and Discussion.

* $pI_{50} = -\log_{10}$ (reagent concentration giving 50% inhibition).

the completion of this work, such a rearrangement for nitrenophenols was suggested by Katzenellenbogen *et al.* [14].

To summarize, we have shown that although diazidodialkylquinones inhibit electron transport in chloroplasts, they are unsuitable as photoaffinity labels. Both the reduced and oxidized quinones can rearrange to unreactive species. Our experimental demonstration that little or no covalent attachment occurs when a radioactive azidoquinone is irradiated with chloroplasts supports the corresponding conjecture of Oettmeier *et al.* [4].

I thank Doctors J. R. Knowles, R. P. Levine, and J. Li for advice. This work was supported by NIH and NSF grants to Dr. Knowles.

[1] A. Trebst, E. Harth, and W. Draber, Z. Naturforsch. **25 b,** 1157 (1970).
[2] J. Li and H. Bayley, Brookhaven Symposium in Biology, No. 28 (J. M. Olson and G. Hind, eds.), p. 362 (1976).
[3] W. Oettmeier, S. Reimer, and A. Trebst, 4th Int. Congress on Photosynthesis, Reading, England, 1977, Abstracts p. 279.
[4] W. Oettmeier, S. Reimer, and K. Link, Z. Naturforsch. **33 c,** 695 (1978).
[5] H. W. Moore and H. R. Shelden, J. Org. Chem. **33,** 4019 (1968).
[6] L. I. Smith and J. Nichols, J. Am. Chem. Soc. **65,** 1739 (1943).
[7] K.-Y. Zee-Cheng and C. C. Cheng, J. Med. Chem. **13,** 264 (1970).
[8] M. Avron, Anal. Biochem. **2,** 535 (1961).
[9] R. P. Levine and R. M. Smillie, Proc. Nat. Acad. Sci. USA **48,** 417 (1962).
[10] H. Bayley and J. R. Knowles, Methods Enzymol. **46,** 69 (1977).
[11] A. Klip and C. Gitler, Biochem. Biophys. Res. Commun. **60,** 1155 (1974).
[12] H. W. Moore, Chem. Soc. Rev. **2,** 415 (1973).
[13] J. R. Knowles, Acc. Chem. Res. **5,** 155 (1972).
[14] J. A. Katzenellenbogen, H. N. Myers, H. J. Johnson, R. J. Kempton, and K. E. Carlson, Biochemistry **16,** 1964 (1977).

Verantwortlich für den Inhalt: A. Klemm
Satz und Druck: Konrad Triltsch, Würzburg