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SHORT COMMUNICATION

# INHIBITION OF ELECTRON TRANSPORT IN *PROTOTHECA ZOPFII*

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**Abstract**—The effects of a number of inhibitors on the respiration of the colourless alga, *Prototheca zopfii*, have been studied, and twenty-six compounds related to rotenone tested as inhibitors of NADH oxidase. The results are compared with those obtained with other algal systems and the mammalian respiratory chain.

## INTRODUCTION

IN A previous paper,[1] it was reported that the mitochondrial electron transport chain of the colourless alga, *Prototheca zopfii*, contains the classical cytochrome components and ubiquinone. It has been suggested that the main pathway of electron transport in many algae may differ somewhat from that observed in animal cells[2] and in order to confirm previous results obtained with *Prototheca* the effects of a number of respiratory chain inhibitors were tested on this organism.

## RESULTS AND DISCUSSION

Insensitivity of respiration to CO has been noted, for example in *Chlorella*[2] and *Euglena*,[3] and the significance of cytochrome oxidase in the respiration of *Chlamydomonas* has been questioned on the basis of spectroscopic evidence.[4] Figure 1 shows the marked inhibition by CO of the oxidation of acetate by whole cell suspensions of *Prototheca*. The respiration of *Prototheca*, unlike some plant systems,[5] is also cyanide-sensitive; $10^{-4}$M-cyanide reduced oxygen uptake with acetate as substrate to the endogenous level.

The succinoxidase activity of isolated mitochondria, assayed polarographically,[6] was completely inhibited by $10^{-6}$M-antimycin A, which acts between cytochromes *b* and *c* in the mammalian respiratory chain. Malonate (10 mM), a competitive inhibitor of succinate dehydrogenase completely inhibited succinoxidase with succinate at a concentration of 10 mM. In contrast to results with *Euglena* mitochondria,[7] 2,4-dinitrophenol did not significantly inhibit succinoxidase at concentrations between $10^{-4}$M and $10^{-5}$ M but led to uncoupling of phosphorylation.

[1] D. LLOYD, *J. Gen. Microbiol.* Submitted for publication.
[2] M. GIBBS, *Physiology and Biochemistry of Algae* (Edited by R. A. LEWIN), p. 75. Academic Press, New York (1962).
[3] F. PERINI, J. A. SCHIFF and M. D. KAMEN, *Biochim. Biophys. Acta* **88**, 91 (1964).
[4] B. CHANCE and R. SAGER, *Plant Physiol.* **32**, 548 (1957).
[5] D. P. HACKETT, *Ann. Rev. Plant Physiol.* **10**, 113 (1959).
[6] D. LLOYD, *Biochim. Biophys. Acta.* In press.
[7] D. E. BUETOW and P. J. BUCHANAN, *Biochim. Biophys. Acta* **96**, 9 (1965).

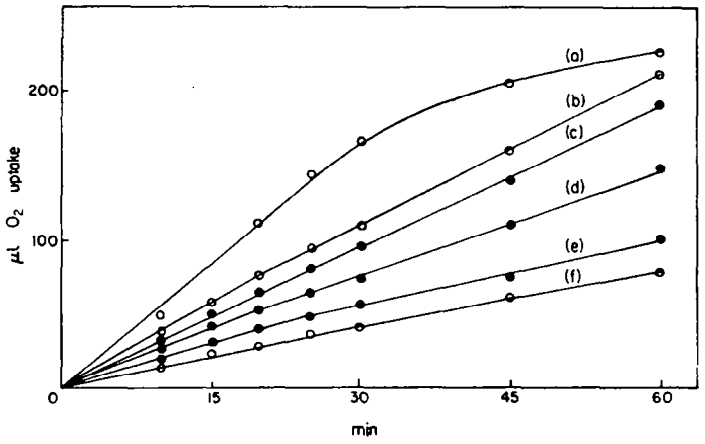



FIG. 1. INHIBITION OF ACETATE OXIDATION BY CO.

The manometer flasks contained 4 mg dry wt of cells in 0·05 M-phosphate buffer (pH 7·2) to a final volume of 2·9 ml with KOH in the centre wells, and after gassing, the reactions were started by tipping in sodium acetate (10 µmoles) from the side arms, except in the endogenous control (f). The gas phases were (a) and (f) air, (b) 19 $N_2$:1 $O_2$, (c) 9 CO:1 $O_2$, (d) 14 CO:1 $O_2$, (e) 19 CO:1 $O_2$. Temperature of incubation: 30°.

The action of a number of inhibitors on the NADH oxidase activity of isolated intact mitochondria is shown in Table 1. Cyanide, azide, antimycin A and amytal are all inhibitory, and the algal system shows a sensitivity to these compounds similar to that observed with the mammalian respiratory chain.

TABLE 1. THE INHIBITION OF NADH OXIDASE BY CYANIDE, AZIDE, ANTIMYCIN A AND AMYTAL

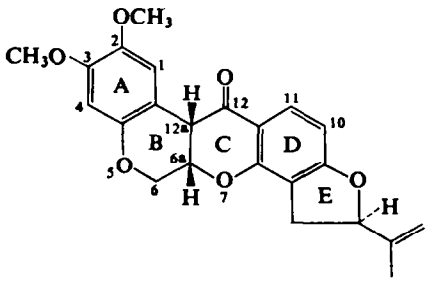
| Compound | Molarity (µmoles/l.) | Inhibition (%) | Compound | Molarity (µmoles/l.) | Inhibition (%) |
|---|---|---|---|---|---|
| Sodium amytal | 1500 | 78 | Antimycin A | 20 | 90 |
| | 1000 | 54 | | 2 | 86 |
| | 400 | 30 | | 0·1 | 86 |
| | 200 | 28 | | 0·02 | 82 |
| KCN | 100 | 90 | Sodium azide | 1000 | 65 |
| | 50 | 83 | | 500 | 59 |
| | 10 | 69 | | 100 | 36 |
| | 5 | 48 | | | |
| | 1 | 26 | | | |

The assay system contained phosphate buffer (pH 7·4) 300 µmoles, NADH 0·4 µmoles, the inhibitor and water to a final volume of 3 ml. The reaction was started by adding 0·05 ml (0·17 mg protein) of mitochondrial suspension and followed by the decrease in extinction at 340 mµ. The control cuvettes contained no inhibitors. Antimycin A was added in 20 µl of ethanol and the control in this case also contained ethanol. Temperature of incubation: 30°.

Rotenone (I) specifically inhibits the oxidation of NADH-linked substrates and the site of action has been localized to the flavin region of the electron transport chain.[8,9] NADH

[8] K. E. ÖBERG, *Exptl Cell Res.* **24**, 163 (1961).
[9] L. ERNSTER, G. DALLNER and G. F. AZZONE, *J. Biol. Chem.* **238**, 1124 (1963).

oxidase activity in *Prototheca* mitochondria is decreased in the presence of retonone although at $3 \times 10^{-7}$M the compound had no effect on succinoxidase activity. A number of compounds related to rotenone were therefore tested as inhibitors of NADH oxidase. The results shown



(I) Rotenone

in Table 2 indicate that several of the rotenoids tested are more powerful inhibitors than rotenone itself while many others are only weakly inhibitory at the concentrations tested. The most active inhibitors are rotenoids with a hydroxyl at C-11 (for chemistry of rotenoids see review of Crombie).[10]

TABLE 2. THE INHIBITION OF NADH OXIDASE BY ROTENONE AND RELATED COMPOUNDS

| Rotenoid | Concentration producing 50% inhibition ($M \times 10^8$) | Rotenoid | Concentration ($M \times 10^6$) | Inhibition (%) |
|---|---|---|---|---|
| (−)-dihydromalaccol | 1·2 | (+)-isorotenone | 1·7 | 30 |
| (−)-sumatrol (11-hydroxy-rotenone) | 1·6 | rotenone-β-oxime | 1·7 | 29 |
| (−)-α-toxicarol (11-hydroxy-deguelin) | 2·8 | racemised rotenol | 1·7 | 28 |
| (±)-isorotenone | 8 | 6a,12a-dihydro-6*H*-rotoxen-12-ol | 2·7 | 15 |
| rotenone | 8·5 | 6a,12a-dihydro-6*H*-rotoxen-12-one | 2·7 | 14 |
| amorphigenin | 9 | (−)-rotenol | 1·7 | 7 |
| derritol | 10 | 6a,12a-dihydro-6,6-dimethyl-6*H*-rotoxen-12-ol | 2·5 | 0 |
| (±)-β-toxicarol | 20 | (−)-dihydroelliptone | 1·9 | 0 |
| dihydrorotenone | 30 | amorphin (the glycoside of amorphigenin) | 1·5 | 0 |
| deguelin | 50 | (±)-α-toxicarol* | | |
| rotenone-α-oxime | 70 | (−)-malaccol (11-hydroxy-elliptone)* | | |
| (+)-epirotenone | 200 | | | |
| epi-hydroxyrotenone | 200 | | | |
| (−)-elliptone | 300 | | | |
| (±)-hydroxy-β-dihydrorotenone | 300 | | | |

* Very inhibitory; low ethanol solubility prevented accurate assay.

The assay conditions were as in Table 1. The inhibitors were added in 20 μl of ethanol and the controls also contained ethanol.

[10] L. CROMBIE, *Fortschritte der Chemie organischer Naturstoffe*, Vol. XXI, (Edited by L. ZECHMEISTER), p. 275. Springer-Verlag, Wien (1963).

The rotoxenols and rotoxenone, consisting of the unsubstituted rotenoid nucleus without ring E, are inactive as are the epimers (+)-isorotenone and (+)-epirotenone. A wide range of activities is exhibited by the other five-ring compounds related to rotenone in which the structure of ring E has been altered. Rotenol (with ring C open) is not inhibitory, while derritol (rings B and C open) is quite active. Thus while some relationships exist between chemical structure and inhibitory activity of the rotenoids it seems that a physical property, such as lipid solubility may contribute to this activity, and these natural products and related compounds might prove useful in further investigations of the exact mechanism of rotenone inhibition.

Burgos and Redfearn[11] have recently tested a number of rotenoids as inhibitors of NADH oxidase of a heart-muscle preparation. In their system rotenone itself was the most active inhibitor. These investigators have found that rotenone has little inhibitory effect on NADH-ferricyanide reductase, an observation that has since been confirmed in mitochondria from *Prototheca*, and they conclude that the site of inhibition is on the oxygen side of the flavoprotein rather than between NADH and the flavoprotein as previously proposed.[8,9]

Thus the effects of respiratory chain inhibitors on electron transport in *Prototheca* are similar to those observed with the mammalian mitochondrial system and confirm that the electron transport chain of this alga is more like the mammalian system than other algal systems which have been studied.[2–4,7]

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[11] J. BURGOS and E. R. REDFEARN, Personal communication, *Biochim. Biophys. Acta*. In press.