DIBROMOTHYMOQUINONE: A NEW INHIBITOR OF MITOCHONDRIAL ELECTRON TRANSPORT AT THE LEVEL OF UBIQUINONE

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1. Introduction

Trebst et al. [1] reported that dibromothymoquinone inhibits photosynthetic electron transport by acting as an antagonist for plastoquinone. Due to the structural and functional similarity of plastoquinone and ubiquinone in chloroplasts and mitochondria respectively, an inhibition of mitochondrial electron transport can also be expected. As reported by Lenaz [2] and confirmed by us, dibromothymoquinone inhibits mitochondrial electron transport both from succinate and NADH-linked substrates. The most interesting feature of this inhibitor however is that in the $CN^-$and/or antimycin blocked respiratory chain, the *b*-cytochromes are specifically oxidized, while the redox state of cytochromes c_1, *c*, *a*, a_3 is not affected. This finding is discussed on the basis of the model of electron transport at phosphorylation site II recently proposed by Wikström and Berden [3].

2. Materials and methods

Dibromothymoquinone was a gift of Dr. Melandri. Rat heart mitochondria were prepared according to ref. [4]. Difference spectra were carried out with a split-beam spectrophotometer designed and constructed in the Johnson Research Foundation, University of Pennsylvania. Oxygen uptake was measured with a Clark-electrode [5].

3. Results

The inhibition of mitochondrial respiration by dibromothymoquinone is shown in fig. 1. The rate of O_2-uptake of freshly prepared rat heart mitochondria respiring on succinate (curve A) or on malate/glutamate (curve B) in state 3 [6] was titrated with dibromothymoquinone. Between 20–25 μM dibromothymoquinone was necessary to obtain 50% inhibition of mitochondrial respiration.

Difference spectra of rat heart mitochondria in the presence and absence of dibromothymoquinone are shown in fig. 2. The base line A was obtained by reducing the mitochondrial respiratory chain with succinate in the presence of CN. Addition of 70 μM dibromothymoquinone to the sample cuvette resulted only in the oxidation of cytochrome *b* at 562 nm (curve B). The other cytochromes (c_1,*c*, *a*,a_3) remained reduced under these conditions.

In the uncoupled respiratory chain CN reduces all cytochromes, except cytochrome b_{566}, which remains largely oxidized [7, 8]. In the presence of antimycin however, this cytochrome *b*-species also becomes reduced [7, 8]. Therefore antimycin has been subsequently added to both cuvettes of the split-beam spectrophotometer in order to reduce also this cytochrome *b*-species. The difference spectrum obtained under these conditions is shown in curve C: (succinate + CN + antimycin) ± dibromothymoquinone. The oxidation of cytochrome *b* is now twice as large and accompanied with a shift of 2 nm to longer

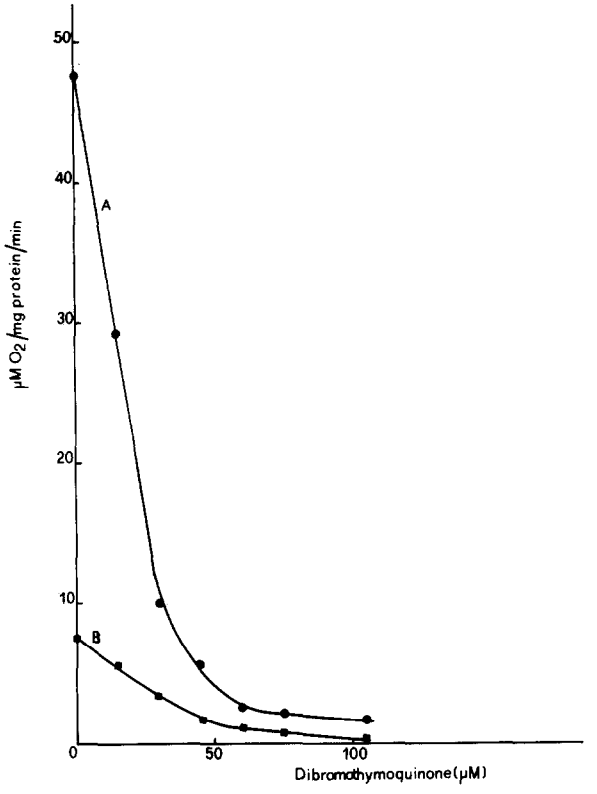


Fig. 1. Inhibition of mitochondrial respiration by dibromothymoquinone. Rat heart mitochondria (1 mg protein/ml) were supplemented with 3 mM P_i and 3 mM ADP in sucrose–Tris buffer (0.25 M sucrose, 10 mM Tris–HCl, pH 7.2). Curve A: Rate of O_2-uptake in the presence of 3 mM succinate and 2 μM rotenone. Curve B: Rate of O_2-uptake in the presence of malate/glutamate (2 mM each).

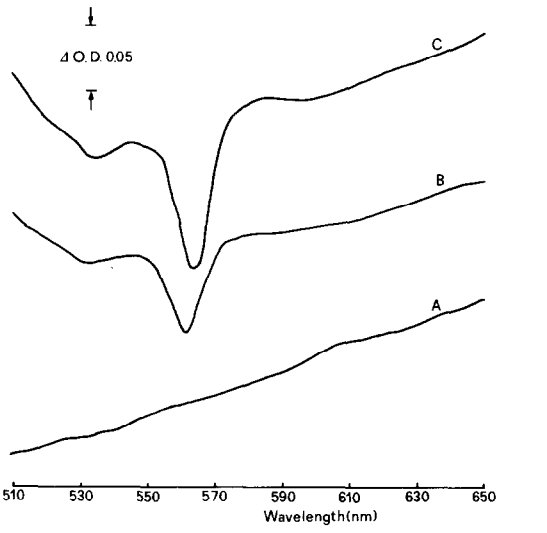


Fig. 2. Dibromothymoquinone induced oxidation of cytochromes *b*. Difference spectra of rat heart mitochondria (2.5 mg protein/ml) in state 3 were monitored with a split-beam spectrophotometer. Curve A: Base line (= 3 mM succinate, 5 mM CN, 3 mM P_i, 6 mM ADP and 2.5 mg mitochondrial protein/ml in each cuvette). Curve B: Base line ± 70 μM dibromothymoquinone. Curve C: The same as curve B, after the addition of 0.5 μg antimycin/mg protein to both cuvettes.

wavelengths (564 nm) indicating that this inhibitor prevents specifically the reduction of both *b*-cytochromes having absorption maxima at 562 nm and 566 nm. The redox states of cytochromes c_1, *c*, *a*, a_3 are not affected under these conditions by this inhibitor.

In different experiments with beef heart mitochondria, mitochondrial membrane fragments and succinate cytochrome *c* reductase [9] the presence of this inhibitor resulted always in a specific, large oxidation of cytochromes *b*, whatever the sequence of additions had been. Furthermore, the specific oxidation of cytochromes *b* could be reversed upon addition of phenoxymethylsulfate.

4. Discussion

The inhibition of mitochondrial electron transport by dibromothymoquinone from both the succinate and the NADH pathways, the induction of cytochromes *b* oxidation and the structural similarity with ubiquinone makes it most likely that the site of inhibition is at the branching point of the respiratory chain on the substrate side of cytochromes *b* at the level of ubiquinone.

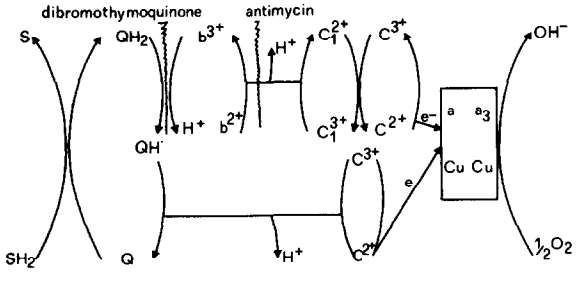
Since the absorption spectrum of this inhibitor is nearly identical to that of ubiquinone, it could experimentally not be decided whether the oxidation of *b*-cytochromes induced by this inhibitor is accompanied by a reduction of ubiquinone. Though rather unlikely it can therefore not be excluded that dibromothymoquinone exhibits separate effects on succinate and NADH dehydrogenase.

Furthermore it could not yet be clearly decided whether the oxidized or reduced form is inhibitory. When dibromothymoquinone was reduced by sodium borohydride it became rapidly auto-oxidized by air. If

dibromothymoquinol were formed by interaction with the mitochondrial respiratory chain its rapid auto-oxidation should result in H_2O_2 or O_2^- radical formation. Since instead, we observed an inhibition also of the endogenous rate of O_2^- radical and H_2O_2 formation of mitochondria by this inhibitor, it is most likely that the oxidized form is the inhibiting species.

The most puzzling finding however, which needs to be explained, is the specific oxidation of just the *b*-cytochromes.

In a CN^--blocked respiratory chain, reduced by succinate or NADH-linked substrates, all electron carriers should become highly reduced if they are in equilibrium with each other [8]. It is known however, that in the uncoupled respiratory chain blocked by CN, succinate or NADH fail to reduce the cytochrome b_{566} unless special conditions are employed [7, 8]. This is explained by the existence of an accessibility barrier (structural or kinetic) [10] for this *b*-species. From the results reported above it seems that this 'accessibility barrier' is extended by dibromothymoquinone also to cytochrome b_{562}. It is generally agreed now that the b-cytochromes are in the main-path of the respiratory chain [7, 8]. Their specific oxidation induced by this inhibitor without affecting the redox states of cytochromes c_1,c,a,a_3 is highly indicative to us that nevertheless some electrons can bypass the *b*-cytochromes keeping the high-potential cytochromes c_1,c,a,a_3 reduced. The action of this inhibitor can be explained by (and indeed is a strong support for) the model of electron transport as proposed recently by Wikström and Berden [3]:



According to these authors the two electrons from ubiquinol are reducing cyt. *c* on two separate pathways, only one of which contains cytochromes *b* and can be inhibited by antimycin, while the second electron reaches cyt. *c* directly bypassing the *b*-cytochromes. The specific oxidation of both *b*-cytochromes in the presence of CN and/or antimycin induced by dibromothymoquinone can easily be explained on the basis of this model, if one assumes that the site of inhibition is in the upper pathway between ubiquinol and cytochromes *b*, while the high potential cytochrome species c_1,c,a,a_3 are still sufficiently kept reduced by the semiquinol radicals through the lower pathway of this scheme. It should be pointed out that most inhibitors of mitochondrial electron transport block the respiratory chain at a phosphorylation site and thus separate the respiratory chain into different pools of iso-potential electron carriers. Dibromothymoquinone however, seems to be the first inhibitor blocking electron transport *not* at a phosphorylation site, allowing equilibration of electron carriers of different potential pools.

Acknowledgements

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References

[1] Trebst, A., Harth, E. and Draber, W. (1970) Z. Naturforsch. 25 B, 1157–1159.
[2] Lenaz, G., Baccarini Melandri, A., Bertoli, E., Masotti, L. and Melandri, B. A. Abstract presented at the First Meeting of the Italian Bioenergetic Group, Rome, Italy (1973).
[3] Wikström, M. K. F. and Berden, J. A. (1972) Biochim. Biophys. Acta, 283, 403–420.
[4] Pande, S. V. and Blachaer, M. C. (1971) J. Biol. Chem. 246, 402–411.
[5] Estabrook, R. W. (1967) Methods in Enzymology, vol. X, 41–47.
[6] Chance, B. and Williams, G. R. (1956) Advances in Enzymology, vol. 17. Nord, F. F., ed. Interscience Publishers, New York, p. 65.
[7] Slater, E. C. (1973) Biochim. Biophys. Acta, 301, 129–154.
[8] Wikström, M. F. K. (1973) Biochim. Biophys. Acta, 301, 155–193.
[9] Erecińska, M., Oshino, R., Oshino, N., and Chance, B. (1973) Arch. Biochem. Biophys. 157, 431–445.
[10] Lee, I. Y. and Slater, E. C. (1972) Biochim. Biophys. Acta 283, 223–233.