DIBROMOTHYMOQUINONE : AN INHIBITOR OF AEROBIC ELECTRON TRANSPORT AT THE LEVEL OF UBIQUINONE IN *ESCHERICHIA COLI*

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Received 31 January 1975

1. Introduction

Dibromothymoquinone (DBMIB)** inhibits photosynthetic [1] and mitochondrial [2] electron transport by acting as an antagonist for benzoquinone function in both systems. Since there are few site-specific inhibitors of electron transport in *Escherichia coli*, other than KCN [3], we have investigated the effect of DBMIB on the growth and respiratory chain of aerobic cultures of this bacterium. In this paper we demonstrate that DBMIB inhibits: a) growth of the organism on a non-fermentatable carbon source, b) NADH, D-lactate and D,L α-glycerophosphate oxidase activities together with NADH- and D-lactate – DCPIP reductase activities of membrane particles, and c) NADH-dependent cytochrome *b* reduction. In addition we show that in the presence of membrane particles, substrate, and KCN, DBMIB catalyzes a rapid oxygen uptake activity that is apparently cytochrome independent but can result in the partial oxidation of the *b*-type cytochromes. We conclude that DBMIB acts as an antagonist of ubiquinone function in the aerobic respiratory chain of *E. coli* on the substrate side of the cytochromes [4] but, because the compound can undergo rapid reduction and auto-oxidation under certain conditions, its general application as an inhibitor of electron transport is limited.

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** *Abbreviations*: DBMIB, dibromothymoquinone (2,5 dibromo-3-methyl-6-isopropyl-*p*-benzoquinone); DCPIP, 2,6,dichlorophenol-indophenol.

2. Materials and methods

E. coli strain EMG-2 (prototroph) was grown in a mineral salts medium [5] containing glycerol (0.5% w/v) and vitamin-free casamino acids (0.1% w/v) under conditions of vigorous aeration in a 10-litre fermentor vessel [4], unless otherwise stated. Strain A1004a (*hem* A^-) is a cytochrome-deficient mutant of *E. coli* and was grown as described previously [6]. Particles were prepared from both strains, by the procedure of Haddock and Downie [7], in a buffer containing 10 mM HEPES–KOH (pH 7.5), 300 mM KCl and 5 mM $MgCl_2$.

Oxidase activities [7], NADH-$K_3Fe(CN)_6$ NADH-DCPIP and D-lactate-DCPIP reductase activities [6], protein determinations [6] and low temperature spectroscopy [8] were measured or performed as described in previous publications from this laboratory.

DBMIB was a generous gift from Dr W. Draber (Bayer AG, Wuppertal, W. Germany) and ubiquinone$_1$ was a generous gift from F. Hoffman - La Roche, Basel, Switzerland. All other reagents were obtained from commercial sources and of the highest available purity.

3. Results and discussion

The data in fig.1 indicate that DBMIB (0.5 mM) inhibits growth and ultimately causes lysis of *E. coli* in a mineral salts medium with glycerol as carbon source but has comparatively little effect when growth is dependent upon a fermentable carbon source. The presence of 0.1 mM DBMIB temporarily arrested expo-

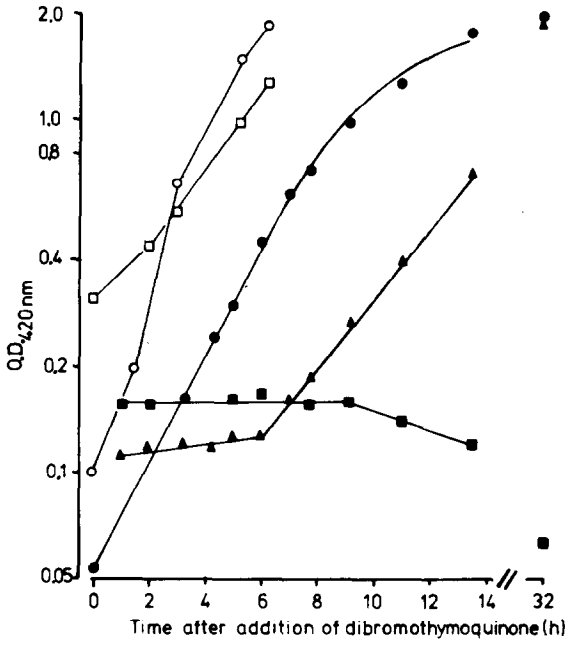


Fig.1. Effect of dibromothymoquinone on growth of *E. coli* Strain EMG-2. Cells from a 24 hr stationary phase culture of *E. coli* (grown with the appropriate carbon source in the absence of DBMIB) were incubated into a mineral salts medium [5] containing either glucose (0.5%, w/v; open symbols) or glycerol (0.3% w/v; closed symbols) in the absence of (-○-, -●-) or presence of DBMIB (-▲-, 0.1 mM; -□-, -■-, 0.5 mM). Bacterial growth was measured turbidimetrically at 420 nm.

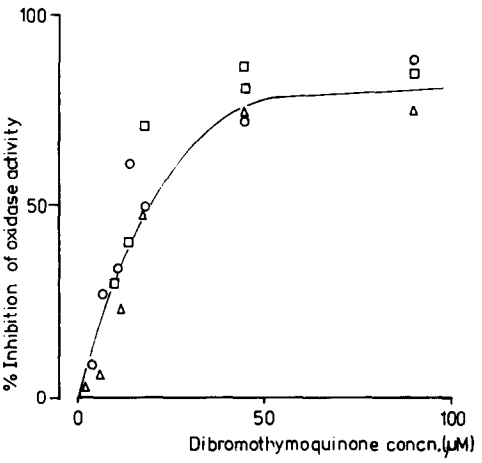


Fig.2. Effect of dibromothymoquinone on oxidase activities of particles from *E. coli*. Strain EMG-2 was grown and particles prepared as indicated in Materials and methods. Oxidase activities were assayed polarographically at 30°C in the presence of NADH (0.2 mM, -○-) at 0.15 mg protein/ml, D-lactate (5 mM, -△-) at 0.75 mg protein/ml, or D,L-α-glycerophosphate (5 mM, -□-) at 0.75 mg protein/ml. The % inhibition of oxidase activity was calculated from a comparison of the rate of substrate oxidation before and 2 min after the addition of various concentrations of DBMIB. The uninhibited rates of oxidation in ng atoms O/min/mg protein were: NADH – 658; D-lactate – 120; and D,L-α-glycerophosphate – 68.

nential growth of the culture on glycerol, for approximately three doubling times, after which growth resumed. Since growth on glycerol, unlike glucose, requires a functioning respiratory chain [9] it appears that DBMIB has some specific effect on electron transport rather than some other cellular process.

This conclusion is supported by the data of fig.2 which show that DBMIB inhibits the oxidation of NADH, D-lactate and D,L-α-glycerophosphate by membrane particles. Half-maximal inhibition of all oxidase activities was achieved with 15–25 μM DBMIB, and the maximal inhibition attained was never greater than 85%. DBMIB (50 μM)-inhibited NADH oxidase activity was not stimulated by the subsequent addition of an excess of ubiquinone$_1$ (280 μM), though DBMIB-inhibited non-cyclic electron flow in chloroplasts has been shown previously to be competitively reversed by plastoquinone [10]. Low concentrations of DBMIB (15–25 μM) caused a stimulation (10–40%) in the NADH- and D-lactate – DCPIP reductase activities but higher concentrations (100 μM) resulted in 100% inhibition of these rates. DBMIB (1 mM) did not inhibit NADH-$K_3Fe(CN)_6$ reductase activity though, again, lower concentrations (0.1 mM) resulted in a 50% stimulation in the apparent rate. Thus the site of action of DBMIB is on the oxygen side of the junction of the various dehydrogenases with the respiratory chain, and, of the two acceptors used to assay dehydrogenase activity, DCPIP interacts with the respiratory chain on the oxygen side, and $Fe(CN)_6^{3-}$ on the substrate side of the inhibitor site.

DBMIB also inhibits NADH-dependent cytochrome *b* reduction in *E. coli* membrane particles (fig.3, compare traces A and B) indicating that DBMIB inhibition of electron transport occurs at the level of ubiquinone on the substrate side of the cytochromes as shown in scheme 1 [3,4]. However, the addition of DBMIB to KCN-inhibited particles results in the partial

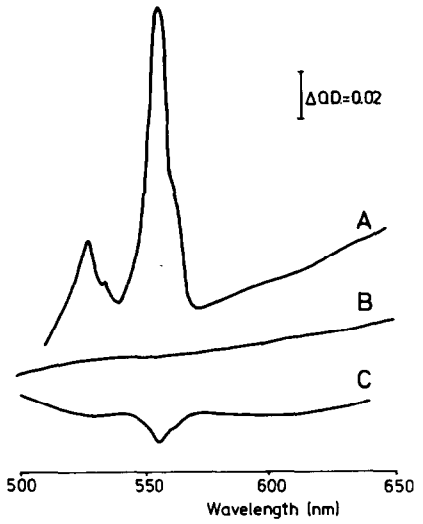
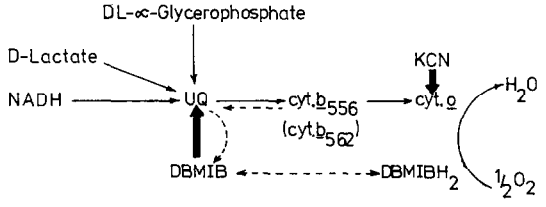


Fig.3. Effect of dibromothymoquinone on cytochrome *b* reduction in particles from *E. coli*. Difference spectra of a particle preparation of *E. coli* strain EMG-2 were recorded at 77°K. Curve A: NADH(1 mM) + H_2O_2 (2 mM) in test cuvette, H_2O_2 (2 mM) in reference cuvette, frozen after 2 min. Curve B: as curve A but DBMIB (300 μM) in test cuvette. Curve C: particles were incubated for 2 min with NADH (1 mM) and KCN (5 mM), H_2O_2 (2 mM) was then added and a sample transferred to the reference cuvette; DBMIB (300 μM) was added to the remainder which was then transferred to the test cuvette and the cuvette assembly frozen. Protein concentrations were 48 mg/ml (curves A and B) or 58 mg/ml (curve C).



Scheme 1. Site of interaction of dibromothymoquinone with the respiratory chain of aerobically grown *E. coli*. Heavy lines indicate site of interaction of inhibitors, continuous lines indicate flow of reducing equivalents during normal electron transport and dotted lines indicate the possible pathway of reducing equivalents in the presence of DBMIB and KCN. Reduced DBMIB ($DBMIBH_2$) oxidation is depicted as resulting in water. however, as the product of the auto-oxidation have not been determined, this could be H_2O_2 or O_2^- radical [2].

reoxidation of about 10% of the *b*-type cytochromes (fig.3, trace C). An analogous effect has been observed previously in mitochondria [2] and explained in terms of the model of electron transport proposed by Wikström and Berden [11]. Based on the rapid rates of reduced DBMIB auto-oxidation [2], we propose that this effect can be more simply explained on the assumption that DBMIB is both an inhibitor of and an auto-oxidizable redox mediator with the respiratory chain (see scheme 1). If correct this proposal readily explains the DBMIB-dependent partial reoxidation of *b*-type cytochromes of similar redox potential to ubiquinone in the presence of KCN, the inability of DBMIB to cause complete inhibition of oxidase activity and, depending upon its concentration, the ability of DBMIB to both stimulate and inhibit various dye reductase activities.

The interactions of DBMIB with the respiratory chain, however, may be even more complex. It can be shown that the addition of DBMIB to KCN-inhibited cytochrome-containing membrane particles of *E. coli* in the presence of substrate results in rapid O_2 consumption (fig.4, trace A). This observation can be explained

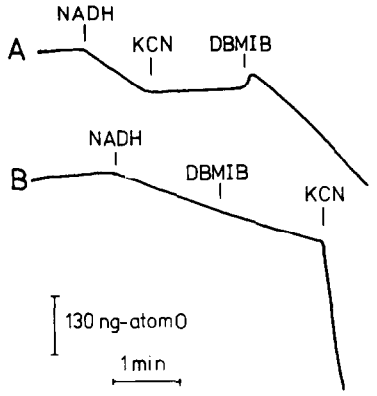


Fig.4. Dibromothymoquinone stimulation of KCN-inhibited NADH oxidase activity in particles from *E. coli*. Oxidase activities were assayed polarographically using in curve A, particles (0.15 mg protein/ml) from strain EMG-2 (cytochromes present) or, in curve B, particles (0.5 mg protein/ml) from strain A1004a (cytochrome deficient). Other additions were NADH (0.2 mM), KCN (5 mM) and DBMIB (140 μM). The rates of oxygen consumption in ng atoms O/min/mg protein were: after NADH addition 705 (curve A) or 48 (curve B) and after the subsequent addition of both KCN and DBMIB 1200 (curve A) or 5300 (curve B). These latter rates were only observed in the simultaneous presence of particles, NADH, KCN and DBMIB.

in accordance with the proposals in scheme 1 since, in the presence of KCN, NADH oxidation could only result in O_2 utilization by the additional presence of a redox mediator, capable of undergoing reduction and auto-oxidation, interacting with the respiratory chain on the substrate side of the site of inhibition by KCN. However, a similar result is obtained with particles prepared from a cytochrome deficient strain of *E. coli* (fig.4, trace B) which have been shown previously to contain no detectable cytochromes, normal concentrations of ubiquinone but high concentrations of menaquinone [6,9]. Thus the possibility exists that KCN stimulates endogenous quinone(s) – DBMIB interaction(s) directly by some, as yet, unexplained mechanism rather that indirectly by inhibition of the conventional cytochrome oxidase activity.

From this study it is clear that DBMIB may be used as a site specific inhibitor of electron transport at the level of ubiquinone in aerobically grown *E. coli*, though care must be taken in the interpretation of data obtained from its use because of its ability to serve as an auto-oxidizable redox mediator with the respiratory chain. Perhaps the most promising application of ubiquinone analogues, like DBMIB, and also of menaquinone analogues, such as 2-hydroxy-3(cyclohexylpropyl)-1-4-naphthoquinone (an inhibitor of aerobic growth of *Bacillus megaterium* but not *E. coli* [12]), whose use as inhibitors of electron transport is restricted because of their auto-oxidation properties, is in the isolation of analogue-resistant mutants. Whilst many of such mutants will no doubt have different permeability properties through alterations to the cell wall, the characterization of others should offer a complementary approach to previous investigations using mutants defective in their ability to synthesize either ubiquinone [3] or menaquinone [13] in the study of quinone function in electron transport.

Acknowledgements

This work was generously supported by the Science Research Council through a Post-Doctoral Research Fellowship (to R.K.P.) and grant (S/RG/58518). We should like to thank Mrs Yvonne Begg and Mrs Cynthia Greenwood for skilled technical assistance.

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