EFFECT OF DIBROMOTHYMOQUINONE ON SUCCINATE-LINKED REACTIONS IN *ESCHERICHIA COLI*

Raymond L. HOUGHTON, Robert J. FISHER and D. RAO SANADI
Department of Cell Physiology, Boston Biomedical Research Institute, 20 Staniford Street, Boston, Massachusetts 02114, USA

Received 12 July 1976

1. Introduction

Mitochondrial [1] and photosynthetic electron transport [2–4] have both been shown to be sensitive to the ubiquinone analogue dibromothymoquinone (DBMIB). More recently it was demonstrated to be not only an inhibitor at the level of ubiquinone in *Escherichia coli* [5,6] but that it could also function as a redox mediator with the respiratory chain. It has not, however, been ascertained whether DBMIB exerts different effects on the dehydrogenases in *E. coli.* In this paper evidence is presented that DBMIB exhibits significant differences in inhibiting the oxidative reactions in *E. coli,* and that DBMIB is a potent inhibitor in the region of succinate dehydrogenase. Recent studies also show that DBMIB can act at the level of the membrane-bound ATPase [6]. Here we extend this observation by demonstrating that this quinone analogue inhibits at the level of respiration and ATP-linked $NAD(P)^+$ transhydrogenase. The effect of other quinone analogues on oxidase activities in *E. coli* membranes is also studied.

2. Materials and methods

E. coli strain W6 (proline auxotroph derived from ATCC9637) was grown in 500 ml of a modified M9 mineral salts medium [7] in 2 liter shake flasks using 30 mM sodium succinate as sole carbon source. Cells were isolated in the exponential phase of growth. Respiratory particles were prepared as described previously [8]. Respiratory chain-linked activities were monitored by the uptake of oxygen measured polarographically at 37°C, in 1 ml of 50 mM potassium phosphate buffer, pH 7, in the presence of 5 mM NADH or 20 mM D-lactate (lithium salt) or 20 mM succinate. Dehydrogenases were measured using ferricyanide as acceptor, in a 3 ml assay medium containing 300 μmol potassium phosphate, pH 7.8, 6 μmol potassium cyanide and NADH, sodium succinate or lithium D-lactate at 7.5, 50 and 30 μmol respectively. The reactions were initiated with acceptor after preincubation at 37°C for 5 min with the inhibitor. Pyridine nucleotide transhydrogenase in *E. coli* energized by NADH oxidation or ATP hydrolysis was measured as before [8], but in the absence of dithiothreitol. Benzoquinone and napthaquinone analogues were obtained from Aldrich Chemical Co. and DBMIB was a gift from Dr A. Trebst.

3. Results and discussion

Figure 1 compares the sensitivity of succinate oxidase activity to that of D-lactate and NADH oxidases. Fifty percent inhibition levels (K_{50}) for D-lactate and NADH oxidases were in the range of 500–600 nmol/mg whereas for succinate the level was 5–10 nmol/mg. These observations led to a re-evaluation of the suggestion [5] that the inhibitory effect of DBMIB is a ubiquinone site common to all pathways and to the possibility that it may exhibit different inhibition patterns on each of the dehydrogenases. To further investigate this possibility we studied the effect of DBMIB on both NADH and succinate dehydrogenases using ferricyanide as acceptor.

Figure 2 shows that in *E. coli* particles DBMIB had little or no effect on D-lactate or NADH-ferri-

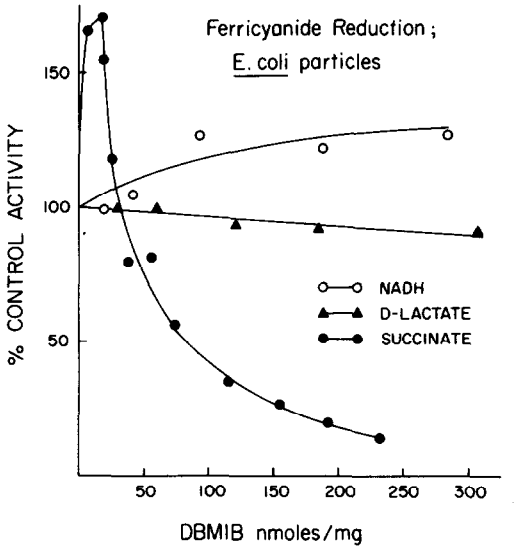

Fig.1: Effect of DBMIB on oxidase activities in succinate grown *E. coli*. Oxidases were measured polarographically at 37°C in the presence of 0.31 mg protein. ▲–▲ D-lactate (20 mM); ○–○ NADH (5 mM); X–X succinate (20 mM). The uninhibited rates were 126, 348 and 420 ngatoms 0 per min/mg for D-lactate, NADH and succinate, respectively.

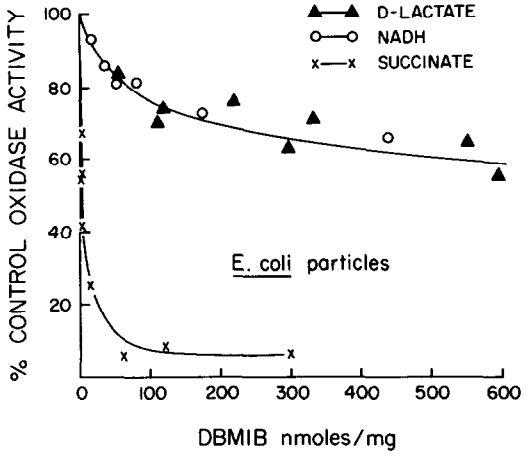

Fig.2: Effect of DBMIB on ferricyanide-reductase activities in succinate grown *E. coli*. Activities were measured as described in the Materials and methods section in the presence of 0.86 mg protein in the case of succinate and D-lactate activities and 0.34 mg in the case of NADH. ○–○ NADH (2.5 mM); ▲–▲ D-lactate (10 mM); ●–● succinate. The uninhibited rates were 1.89, 0.054 and 0.24 μmol ferricyanide reduced per min/mg, respectively.

cyanide reductase activities at concentrations where the succinate-linked activity was almost totally inhibited. However, in the range where succinate oxidase activity was inhibited, the succinate dehydrogenase was stimulated 30–50%. This phenomenon is probably related to the control of succinate dehydrogenase activity by the oxidized to reduced quinone ratio (Q/QH_2) as reported by other workers [9] in mitochondrial systems. The D-lactate-$Fe(CN)_6^{3-}$ reductase activity was insensitive to DBMIB in the range where the succinate-linked activity was completely inhibited pointing to possible differences between these two pathways.

Studies with other benzoquinones showed that several halogenated derivatives inhibited succinate oxidase activity in the concentration range of DBMIB. These same quinones, however, stimulated NADH oxidase and had little effect on D-lactate oxidation. Two such quinones were 2,6-dibromo-3,5-dimethoxy-*p*-benzoquinone and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and their effects are listed in table 1. Similar to DBMIB, both these quinones inhibit succinate-$Fe(CN)_6^{3-}$ reductase activity but were without effect on the NADH-$Fe(CN)_6^{3-}$ reductase. Stimulation of the succinate-linked activity at low levels of these quinones was not apparent. K_{50} values for succinate-$Fe(CN)_6^{3-}$ reductase were at 20 and 15 nmol/mg for 2,6-dibromo-3,5-dimethoxy-*p*-benzoquinone and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone respectively.

Other compounds which inhibit electron transport such as HQNO and piericidin A were without effect on the succinate-$Fe(CN)_6^{3-}$ reductase activity. These same inhibitors, however, inhibit succinate oxidase activity at levels ten times higher than for NADH oxidase (table 1). D-Lactate oxidase is also inhibited in the same range as succinate oxidase (data not shown).

The sensitivity of the succinate oxidase system to napthaquinones was considerably lower than for substituted benzoquinones (table 1). Many of these derivatives stimulated NADH oxidation, presumably by mediating in the electron transport chain. 2-Hydroxy-3-(w-cyclohexyloctyl)-1,4-napthaquinone, however, was a more effective inhibitor of NADH oxidase than succinate or D-lactate oxidase. K_{50} values were 35, 220 and >300 nmol/mg respectively for these three pathways.

Table 1
Effect of quinone analogues on oxidase activities in *E. coli*

Quinone	Concentration (nmol/mg)	% Control oxidase activity	
		NADH	Succinate
Juglone	24	134	80
	42	162	62
	83	166	50
2-ethylthio-3-methyl	38	131	104
napthaquinone	76	143	102
	190	155	101
2,3-dichloronapthaquinone	38	129	36
	76	126	25
	115	132	20
2,3-dichloro-5,6-dicyano-	8	–	66
1,4-benzoquinone	16	–	45
	41	–	12
	201	104	–
	268	131	–
	335	141	9
2,6-dibromo-3,5-dimethoxy-	6	–	6
p-benzoquinone	13	–	57
	51	–	27
HQNO	3.2	77	–
	6.3	64	89
	16	35	89
	31	23	75
Piericidin A	1.2	75	–
	2.4	–	87
	3.6	54	–
	4.8	–	75
	6.0	42	–
	7.2	–	69
	8.4	30	–

In *E. coli* the measurement of $NAD(P)^+$ transhydrogenase driven by the oxidation of succinate is inherently difficult because of the lack of a specific inhibitor such as rotenone in the region of NADH dehydrogenase. One can, however, measure the transhydrogenase driven by the oxidation of NADH and study the stimulatory effect of succinate or ATP and Mg^{2+} on this activity [8]. Figure 3 shows the inhibitory effect of DBMIB on the $NAD(P)^+$ transhydrogenase activity. In the presence of ATP and Mg^{2+} the activity is inhibited in the same range as the NADH-driven activity. The presence of succinate, however, tends to lower the titer for DBMIB, as expected because of the high sensitivity of the succinate dehydrogenase segment of the respiratory chain to DBMIB. The levels are still higher than for the succinate-$Fe(CN)_6^{3-}$ reductase activity due to the continuing involvement of the transhydrogenase energized by NADH oxidation.

DBMIB was also demonstrated to be an inhibitor of ATP-dependent reduction of NAD^+ by succinate and succinate-driven transhydrogenase in beef heart submitochondrial particles. K_{50} values were 25 and 60 nmol/mg respectively corresponding almost exactly to the levels of DBMIB required to inhibit NADH and succinate oxidase, respectively. At high levels of the quinone a cooperative inhibition of ATP-driven

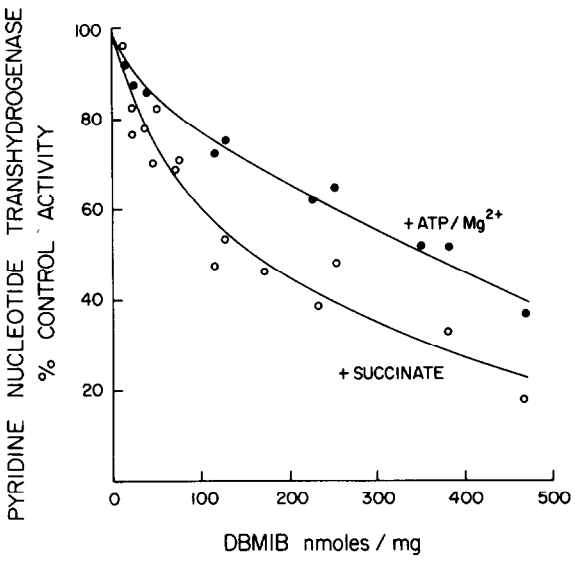

Fig.3: Effect of DBMIB on pyridine nucleotide transhydrogenase activities in *E. coli*. Respiration (NADH)-driven transhydrogenase was measured as described in the Materials and methods section in the presence of either succinate or ATP and Mg^{2+}. 0.22 mg protein were used in each assay. o–o succinate; •–• ATP and Mg^{2+}. Basal rate in the absence of either succinate or ATP and Mg^{2+} was 37 nmol NADPH formed per min/mg. The uninhibited rates in the presence of succinate or ATP and Mg^{2+} were 86 and 153 nmol NADPH formed per min/mg, respectively.

transhydrogenase could also be obtained (data not shown).

Previous studies [5] have shown that NADH and D-lactate reduction of DCPIP in *E. coli* particles were stimulated by 15–25 μM DBMIB but totally inhibited by higher levels. Therefore, in these two pathways, DCPIP interacts with the respiratory chain on the oxygen side of the DBMIB inhibition site and $Fe(CN)_6^{3-}$ on the substrate side. In contrast, the succinate-$Fe(CN)_6^{3-}$ reductase activity is strongly inhibited by DBMIB but is insensitive to HQNO and piericidin A. The inhibition characteristics with these three inhibitors are consistent with an electron transport chain proposed by Cox et al. [10]. By investigating the redox state of various chain components, these authors suggested that in the *E. coli* NADH oxidase pathway a quinone existed on either side of the cytochrome b_1 component. The inhibitory properties of DBMIB reported here are also consistent with this hypothesis and suggest the presence of another quinone site in the succinate oxidase pathway with properties different from the properties of the quinones in the NADH and D-lactate oxidase pathways.

Acknowledgement

This research was supported by grants BMS7503532 from the National Science Foundation and GM13641 from the National Institutes of Health.

References

[1] Loschen, G. and Azzi, A. (1974) FEBS Lett. 41, 115–117.
[2] Bohme, H., Reimer, A. and Trabst, A. (1971) Zeitschrift für Naturforschung 266, 341–352.
[3] Izawa, S., Gould, J. M., Ort, D. R., Felker, P. and Good, N. E. (1973) Biochim. Biophys. Acta 305, 119–128.
[4] Trebst, A., Harth, E. and Drabner, W. (1970) Zeitschrift für Naturforschung 25b, 1157–1159.
[5] Poole, R. K. and Haddock, B. A. (1975) FEBS Lett. 52, 13–16.
[6] Sun, I. L. and Crane, F. L. (1976) Biochem. Biophys. Res. Commun. 68(1), 190–196.
[7] Phares, E. F. (1971) in: Methods in Enzymology, Vol. XVII, pp. 441–476, Academic Press, New York.
[8] Houghton, R. L., Fisher, R. J. and Rao Sanadi, D. (1975) Biochim. Biophys. Acta 396, 17–23.
[9] Ramasarma, R. and Susheela, L. (1974) in: Biomembranes, Architecture, Biogenesis, Bioenergetics and Differentiation, (Packer, L. ed.) pp. 261–278. Academic Press, New York.
[10] Cox, G. B., Newton, N. A., Gibson, F., Snoswell, A. M. and Hamilton, J. A. (1970) Biochem. J. 117, 551–562.