COORDINATED, COENZYME Q REVERSIBLE, 2,5-DIBROMOTHYMOQUINONE INHIBITION OF ELECTRON TRANSPORT AND ATPase IN ESCHERICHIA COLI

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Summary

2,6-dibromothymoquinone (DBMIB) and other coenzyme Q analogs partially inhibit electron transport and the membrane-bound Mg^{++} stimulated ATPase of E. coli membranes. The inhibitions by DBMIB are fully reversed by coenzyme Q_6, and other analogs show partial reversal by coenzyme Q_6. Electron transport reactions inhibited are NADH and lactate oxidase, NADH menadione reductase, lactate phenazinemethosulfate reductase and duroquinol oxidase. The concentrations of DBMIB required are similar for electron transport and ATPase inhibition and inhibitions are all increased by uncouplers. Electron transport and ATPase are not inhibited in a DBMIB insensitive mutant. Soluble ATPase extracted from the membranes does not show DBMIB inhibition under either high or low Mg^{++} conditions. Lipophilic chelators show additional inhibition over DBMIB. It appears that coenzyme Q functions at three sites in E. coli electron transport where ATPase activity is controlled. Coenzyme Q deficient mutants also show decreased electron transport and ATPase activity which is restored by coenzyme Q.

Introduction

2,6-dibromothymoquinone (DBMIB) has been shown to inhibit electron transport in chloroplasts (1,2,3), mitochondria (4) and Escherichia coli membranes (5). In chloroplasts the inhibition is reversed by added plastoquinone whereas in mitochondria and in E. coli membranes the inhibition correlates with proposed site of coenzyme Q function, but the inhibition has not been found to be reversed by coenzyme Q.

We wish to report DBMIB inhibition at three sites of electron transport in E. coli where the inhibition is reversed by added coenzyme Q_6, and no inhibition in strains deficient in coenzyme Q. Furthermore, there is a most remarkable correlation between coenzyme Q reversed DBMIB inhibition of membrane-bound ATPase and the electron transport inhibition.

Methods

E. coli strains used were as follows: AN98 (thi^-, arg^-, met^-, $DBMIB^+$), AN293 (thi^-, arg^-, $ubiB^-$), PU-AN293 (thi^-, arg^-, DBMIB), AT2535 (thi^-, Pyr , his , arg , pur , mal , xyl , strA , tsx , tonA), AN387 ($ubiA^+$) and AN385 ($ubiA^-$). Cells were grown in a manner described by Cox et al. (6). PU-AN293 is a revertant isolated from AN293 (an isogenic strain of AN98), which has normal coenzyme Q content when grown aerobically, but does not grow on succinate. NADH oxidase, NADH menadione reductase and duroquinol oxidase and ATPase assays were as described previously (7,8). Lactate PMS reductase assay contained 0.1 M K(Na) phosphate buffer pH 7.0, 1.0 µmoles phenazine methosulfate (PMS) and 3.0 µmoles D-lactate in a volume of 1.4 ml. Inhibitors were incubated three minutes with enzyme before adding PMS and lactate. DBMIB, coenzyme Q, uncouplers and chelators were added in ethanolic solution. Membranes were prepared as described (8,9), and ATPase was extracted as described by Cox et al. (6).

Results

In addition to the inhibition of NADH oxidase and lactate oxidase (5) DBMIB inhibits the partial reactions NADH menadione reductase, lactate PMS reductase and duroquinol oxidase at similar concentrations (Fig. 1). All of these activities are restored by the addition of coenzyme Q_6 either before or after the addition of DBMIB and are not restored by the addition of similar amounts of α-tocopherol or phylloquinone (Table 1). The uncoupler CCCP increases the extent of inhibition of each

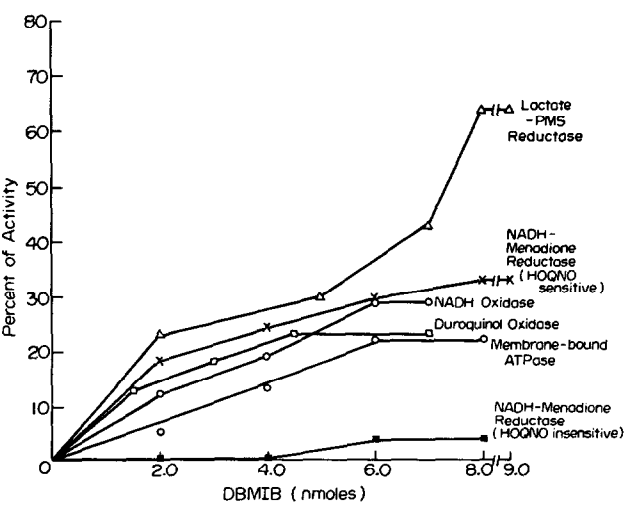

Fig. 1. Inhibition of partial electron transport reactions and ATPase activity by DBMIB.

of these reactions (but does increase the sensitivity to DBMIB at each site) and coenzyme Q_6 reverses completely (Table 1).

The Mg^{++} stimulated ATPase is also partially inhibited by the same concentrations of DBMIB as inhibit the electron transport reactions (Fig. 1), and the inhibition is reversed by coenzyme Q_6. CCCP also causes increased DBMIB inhibition of the ATPase (Table 1).

Table 1

Effects of Coenzyme Q and CCCP on DBMIB Inhibition of Electron Transport and Membrane-bound ATPase Activities

Inhibitors	Percent Inhibition NADH→O_2	Lactate PMS	ATPase	Soluble ATPase
None	0	0	0	0
DBMIB 8.0 nmoles	28	43	23	0
DBMIB 8.0 nmoles + coenzyme Q_6 0.12 µmoles	0	0	0	-
DBMIB 8.0 nmoles + α-tocopherol 0.12 µmoles	24	47	19	-
DBMIB 8.0 nmoles + phylloquinone 0.12 µmoles	32	44	32	-
DBMIB 8.0 nmoles + CCCP 24.0 nmoles	50	55	53	-
coenzyme Q_6 0.12 µmoles	0	2	0	-
CCCP 24.0 nmoles	0	10	15	-
#6967 48.0 nmoles	46	46	40	26
#6967 48.0 nmoles + coenzyme Q_6 0.12 µmoles	16	17	20	19
HOQNO 0.15 µmoles	83	0	0	0
Piericidin A 9.0 nmoles	77	0	-8	-133
Dicoumarol 50.0 nmoles	20	30	-12	4
KCN 30.0 µmoles	99	0	2	-87
DCCD 5.0 nmoles	0	0	50	0

The control rate was in the range of 260-320 nmoles O_2/min/mg protein for NADH oxidase; 100-160 nmoles O_2/min/mg protein for lactate-PMS reductase; 0.5-1.0 µmoles P_i/min/mg protein for ATPase and 0.8-1.4 µmoles P_i/min/mg protein for soluble ATPase. (-) indicates stimulation. DCCD, N,N'-dicyclohexyl carbodiimide; #6967, 3-w-cyclohexyl-pentyl-2-hydroxy-1,4-naphthoquinone; CCCP, carbonyl cyanide-chloro-phenyl-hydrazone; and HOQNO, 2-heptyl-hydroxyquinoline-N-oxide.

Mutant strains which have less (AT2535) or no (AN385) ubiquinone have decreased inhibition by DBMIB of both electron transport (lactate→PMS, NADH→oxygen) and ATPase. We have also isolated a mutant strain PU-AN293 which cannot grow on succinate but has normal coenzyme Q_8 content. In this strain both electron transport and ATPase are insensitive to DBMIB (Table II). The strains which are deficient in coenzyme Q_8 also show lower rates of electron transport and ATPase activity. The activities are increased by added coenzyme Q_6.

When the ATPase is detached from the membrane there is no DBMIB

Table III

Combined Chealtor and DBMIB Inhibition of Electron Transport and ATPase Activities

Inhibitors	Percent Inhibition		
	NADH → O_2	lactate → PMS	ATPase
None	0	0	0
FTFA 14.4 µmoles	50	60	29
FTFA 24.0 µmoles	70	70	38
FTFA 43.2 µmoles	70	78	62
DBMIB 8.0 nmoles	28	33	22
FTFA 43.2 µmoles + DBMIB 8.0 nmoles	95	87	100
FTFA 24.0 µmoles + Coenzyme Q_6 0.12 µmoles	73	67	31
FTFA 14.4 µmoles + CCCP 14.0 nmoles	62	87	65
DBMIB 8.0 nmoles + CCCP 14.0 nmoles	36	55	76
FTFA 14.4 µmoles + DBMIB 8.0 nmoles + CCCP 14.0 nmoles	88	90	50
CCCP 14.0 nmoles	0	13	15

The control rate was in the range of 260-320 nmoles O_2/min/mg protein for NADH oxidase; 100-160 nmoles O_2/min/mg protein for lactate-PMS reductase and 0.5-1.0 µmoles P_i/min/mg protein for ATPase. FTFA, 4,4,4-tri-fluoro-1-(2-furyl)-1,3-butanedione.

inhibition of activity, and coenzyme Q does not stimulate activity.

Other coenzyme Q analogs such as 3-w-cyclohexyl-pentyl-2-hydroxy-1,4-naphthoquinone show greater inhibition of electron transport and ATPase than DBMIB but these inhibitions are only partially reversed by coenzyme Q_6 (Table I). Other compounds which inhibit electron transport, such as 2-heptyl hydroxyquinoline N-oxide, piericidin A, dicoumarol and cyanide do not inhibit ATPase and dicyclohexylcarbodiimide inhibits ATPase without inhibiting electron transport.

We have previously shown that various lipophilic chelators partially inhibit both electron transport and ATPase (7,8). For example, FTFA gives inhibition of up to 50% of each function at similar concentrations. This inhibition is also increased by CCCP but is not reversed by coenzyme Q_6. In the presence of chelators, DBMIB shows additional inhibition in presence or absence of CCCP of both electron transport and ATPase (Table III).

Discussion

The DBMIB inhibition which is reversed by coenzyme Q_6 in the NADH menadione reductase and duroquinol oxidase segments of the electron transport chain is consistant with the proposal by Cox *et al.* (10,11) that coenzyme Q functions at two sites in the NADH oxidase pathway. The DBMIB inhibition of lactate PMS reductase indicates another site of coenzyme Q function in the region between lactate dehydrogenase and cytochrome b. This site differs from the others in that it is not inhibited by piericidin A, HOQNO and bathophenanthroline. DBMIB is also unique in that it causes an inhibition of the membrane-bound ATPase which is reversed by coenzyme Q_6. This inhibition together with the lower ATPase activity in the mutants deficient in coenzyme Q indicates that one or more of the coenzyme Q sites controls the activity of the membrane bound ATPase. We have previously shown that several lipophilic chelators inhibit both electron transport in NADH oxidase and ATPase. Similar effects are now seen in the lactate PMS reductase.

Table II

Relation Between Coenzyme Q Content and DBMIB Inhibition in Various Strains of E. coli

Strain	Coenzyme Q Content	Relative Rate* NADH→O_2		lactate→PMS		ATPase	
	(nmoles Q/ mg protein)	-DBMIB 7 nmoles	+DBMIB 7 nmoles	-DBMIB 8 nmoles	+DBMIB 8 nmoles	-DBMIB 8 nmoles	+DBMIB 8 nmoles
AN98	2.56	1.00 (187)*	0.71	1.00 (92)*	0.67	1.00 (0.54)*	0.77
PU-AN293	2.43	1.00 (171)	0.98	1.00 (58)	1.00	1.00 (0.30)	0.97
AT2535 (aerobiosis)	1.90	1.00 (140)	0.75	1.00 (95)	0.79	1.00 (0.46)	0.59
AT2535 (anaerobiosis)	0.38	1.00 (44)	1.00	1.00 (13)	0.94	1.00 (0.24)	0.96
AN387 ($ubiA^+$)	1.70	1.00 (300)	0.83	1.00 (33)	0.81	1.00 (0.33)	0.67
AN385 ($ubiA^-$)	0.03	1.00 (181)	1.00	1.00 (17)	1.00	1.00 (0.21)	0.93
AN98 ($DBMIB^+$)+Coenzyme Q_6	____	1.00	1.00	0.98	1.00	1.00	1.00
PU-AN293 ($DBMIB^-$)+Coenzyme Q_6	____	1.21	1.11	1.15	1.10	1.10	1.00
AT2535 (aerobiosis)+Coenzyme Q_6	____	1.04	1.02	0.92	0.96	1.02	1.07
AT2535(anaerobiosis)+CoenzymeQ_6	____	1.64	1.18	1.12	0.94	1.46	0.99
AN387 ($ubiA^+$)+Coenzyme Q_6	____	1.06	1.06	1.00	1.00	0.94	0.98
AN385 ($ubiA^-$)+Coenzyme Q_6	____	1.45	1.10	1.30	0.93	1.52	1.09

*Control rates in parenthesis are nmoles O_2/min x mg protein for NADH→oxidase and Lactate→PMS reductase and μmoles P_i/ min x mg protein for ATPase. Relative rates indicate ratio of observed rate/control rate. Coenzyme Q_6 added to 0.12 μmoles where indicated.

The greater extent of inhibition shown by lipophilic hydroxyquinone analogs of coenzyme Q may represent combined coenzyme Q inhibition (reversed by coenzyme Q) and chelator effects (not reversed by coenzyme Q).

Since DBMIB and chelators show additive inhibition of both electron transport and ATPase and the chelator effects are not reversed by coenzyme Q, it appears that both coenzyme Q and a metal site (most likely non-heme iron) are involved at one or more sites in the electron transport chain which controls ATPase activity. These sites would correspond to energy coupling sites proposed by Cox et al. (8) for the NADH oxidase chain and the site which Kaback (12) has shown to energize transport reactions in the lactate PMS reductase segment. It should be noted that the quinone non-heme iron site in the membrane could be a proton generating site for influencing ATP binding to the ATPase.

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