CORRELATION OF THE FUNCTION OF DEMETHYLMENAQUINONE IN BACTERIAL ELECTRON TRANSPORT WITH ITS REDOX POTENTIAL

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1. Introduction

The functions of ubiquinone (Q) and menaquinone (MK) in electron transport have been established with several bacterial species [1–9]. Either Q or MK can serve as a mediator in respiration with low potential donor substrates, whereas succinate respiration requires Q specifically. On the other hand fumarate reduction is specifically mediated by MK.

In this communication the function of demethylmenaquinone (DMK) [10,11] in the electron transport of *Haemophilus parainfluenzae* 1 Fleming is studied which contains DMK together with Q. The method used for this purpose consists in measuring various electron transport activities after reversible extraction of the quinones from the membrane [1,2,12].

2. Methods

Haemophilus parainfluenzae 1 Fleming (NCTC 4101) was grown under aerobic conditions on proteose peptone medium (No 3, Difco) containing 0.35 mM NADH [13]. The bacteria were harvested in the late exponential growth phase, washed twice with 10 mM Tris–HCl buffer containing 0.3 M sucrose, pH 7.2, and disrupted by sonication. The homogenate was centrifuged for 10 min at 14 000 × *g* and the resulting supernatant for 30 min at 125 000 × *g*. The sediment was washed with 0.15 mM KCl and lyophilized at −80°C.

The 'lyophilized particles' were extracted with pentane as described earlier [2,12] to give 'depleted particles'. Reincorporation of the quinones into depleted particles was done by suspending the particles (6 mg protein) in 2 ml pentane containing approx. 1 mg quinone per mg of protein at 0°C. The suspension was shaken for 30 min and evaporated in a rotary evaporator. The residue was resuspended in 0.25 M sucrose.

The contents of Q and DMK were determined after extraction with methanol–petrol-ether as described earlier [1,2]. Using the difference spectrum (KBH_4 reduced versus oxidized) of the extract in ethanol the concentrations of Q and DMK were calculated from the absorbance difference at 280–289 nm and 246–265 nm respectively. The extinction coefficients used were 8.8 [2] and 26.7 $mM^{-1} \times cm^{-1}$ [14] for Q and DMK respectively.

Respiratory activities were measured with a Clark-type oxygen electrode. The particles were suspended in 50 mM phosphate buffer, pH 7.2, at 25°C. Under the same conditions the activity of oxidation of NADH by fumarate was measured as the absorbance decrease at 366 nm caused by the addition of fumarate in the absence of oxygen [1,2].

The standard redox potential at pH 7.0 (E'_0) of DMK was determined polarographically using a Hg-drop electrode. The solvent was isopropanol–H_2O (9:1) containing 0.1 M Tris–HCl, pH 7.2.

DMK was extracted by methanol–petrol-ether from *Haemophilus parainfluenzae* Bossy No 7 Leidy and purified by thin-layer chromatography on silica gel using benzene–chloroform (9:1) as the solvent. MK was obtained by extraction from *Bacillus cereus* Lederle No 5. These bacteria contain the respective

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Table 1
Influence of extraction and reincorporation of quinones on electron transport of *Haemophilus parainfluenzae* 1 Fleming.

	Quinone content		Activity of electron transport		
	DMK	Q	NADH → O_2	succinate → O_2	NADH → fumarate
	(μmole · g protein^{-1})		(μmole · min^{-1} · g protein^{-1})		
Particles:					
Lyophilized	1.20	0.80	99.5	42.1	27.8
Depleted	0.10	0.07	16.3	5.6	1.3
Depleted + DMK			52.0	56.7	23.1
Depleted + Q			49.1	64.0	4.2
Depleted + MK			58.0	6.4	27.1

The growth of bacteria, the preparation of lyophilized and depleted particles as well as the reincorporation of the quinones into depleted particles is described under Methods. The data of this table are typical results of a series of experiments.

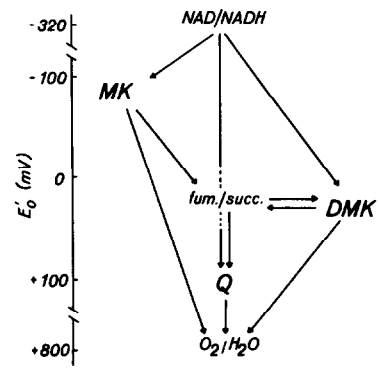
compounds as the only quinones. Q-10 was purchased from Serva GmbH (Heidelberg, Germany).

3. Results and discussion

In the experiment of table 1 the respiratory activities with NADH and succinate as well as the activities of fumarate reduction by NADH were measured as functions of the presence of the quinones in the bacterial particles. On extraction of the particles by pentane after lyophilization 92% of the DMK and 91% of the Q are removed. Concomitantly the three activities of electron transport are decreased down to less than 20% of those of the lyophilized preparation. Reincorporation of DMK into depleted particles causes the restauration both of the respiratory activities (52% with NADH and 135% with succinate) and of the activity of fumarate reduction by NADH (83%). Reincorporation of Q brings about reactivation of the respiratory activities (49% with NADH and 152% with succinate), whereas fumarate reduction is not restored. With MK-reincorporated particles the oxidation of NADH by both oxygen (58%) and fumarate (97%) is reactivated, whereas succinate respiration remains unaffected.

These results indicate that DMK acts as a mediator in respiration with NADH and succinate similar as Q and it serves also as a mediator in fumarate reduction similar as MK. This is confirmed by an equivalent result obtained with *H. influenzae* 18 RAMC Bensted which contains only DMK [15]. Thus in contrast to Q and MK, DMK acts equally well in succinate respiration and in fumarate reduction.

The differences in the capabilities of reactivation of the electron transport by the three quinones are explained on the basis of their redox potentials (scheme 1) rather than by enzyme specificity. The standard redox potential at pH 7.0 (E'_0) of DMK



Scheme 1: Comparison of the redox potentials of the quinones with those of the donors and acceptors of electron transport. The arrows designate the pathways of electron transport which are indicated by the experimental results of table 1.

was measured to be +36 mV and those of Q and MK are +112 mV and −74 mV respectively [16]. From the E'_0 of the fumarate/succinate couple (+30 mV) it is obvious that MK is not reducible by succinate and that reduced Q cannot be oxidized by fumarate. On the other hand reduced MK is well suited as a donor for fumarate and Q as an acceptor for succinate. The E'_0 of DMK which is close to that of the fumarate/succinate couple allows this quinone to serve both as acceptor in succinate oxidation and as donor in fumarate reduction. The finding that each of the quinones can interact in NADH (E'_0 = −320 mV) respiration is also in agreement with the redox potentials.

The enzymes of this bacterium which reduce and oxidize the quinones are not specific for the structures of the quinones. This is evident from the reactivation of the respiratory activities both with Q and DMK. Furthermore, respiration and fumarate reduction with NADH are restored by MK, although this quinone is not synthesized by *H. parainfluenzae* 1 Fleming.

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