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Menaquinone (MK-6) in the sulfate-reducing obligate anaerobe, Desulfovibrio

No reports appear to exist concerning the existence, let alone isolation and characterization, of menaquinones (vitamin K_2) in the genus, Desulfovibrio, which includes a large number of microorganisms exhibiting a strictly anaerobic mode of growth based on reduction of sulfate as terminal H-acceptor There is ample evidence that many anaerobes, even strict anaerobes like Desulfovibrio, show metabolic characteristics more like those of aerobic microorganisms rather than those of fermentative anaerobes Among the photosynthetic bacteria, which include some strict anaerobes, as well as in many facultative anaerobes, there are numerous examples of electron transport systems analogous to those of the mitochondrial system[1–3]. These exhibit all the usual functional components of coupled oxidations, including cytochromes and quinones Following early suggestions[2,4] based on the discovery of *c*-type cytochromes ("cytochromes c_3") in Desulfovibrio[5,6] and with the accumulation of more evidence in recent years[3], it is established that a somewhat truncated electron transport chain, analogous to the mitochondrial system, operates in the chemosynthetic mode of metabolism in Desulfovibrio. Coupling to phosphate esterification occurs with electron transport from either inorganic or organic H-donors to sulfate and other inorganic sulfur compounds as H-acceptors Because the existence of such an electron transport system implies the presence of some type(s) of quinones, as well as the already demonstrated cytochromes, our studies on distribution of quinones in microorganisms have been extended to include two representative strains of Desulfovibrio. We report here our finding that a typical menaquinone (MK-6) exists in relatively large amounts in these obligately anaerobic sulfate reducers

Pure cultures of two strains, *Desulfovibrio gigas* (NCIB 9332) and *Desulfovibrio desulfuricans*, El Agheila Z (NCIB 83), were grown in an "Amsco" fermentor, as described previously[7]. For cultivation of the latter strain, 10 g/l NaCl were added to the culture medium. The washed cells were suspended in 0.1 M Tris–HCl buffer, pH 7.6, and disrupted with a French pressure cell. The suspension was then centrifuged at 4° at 30 000 × *g* for 30 min (Sorvall refrigerated centrifuge). The pellets were collected, accumulated from a number of preparations and stored in the cold (−20°) 50–100 g of the collected residus (wet cell paste) were extracted exhaustively with acetone, the acetone evaporated and the residue taken up in light petroleum for analysis by column and thin-layer chromatography These procedures have been reported elsewhere[8,9]. It was evident from these preliminary experiments that one menaquinone was present. By comparison with authentic samples of MK-6, MK-7 and MK-8 (kindly furnished by Dr. O. Isler, Hofmann-La Roche Co.) it was evident that the menaquinone was most probably MK-6. Measurement of the vitamin K in buffered ethanol[10] allowed an estimate for the content in both strains of 0.4 μmole/g wet weight (1.7 μmoles/g dry weight)

To establish the identity of the menaquinone with MK-6, the natural samples, after repurification by thin-layer chromatography on silica gel G, were compared with MK-6 synthesized in the laboratories of the Institut de Biochimie d'Orsay, using three methods: (a) reverse phase chromatography on paper impregnated with vaseline, prepared by soaking in a 5% solution of vaseline oil (Prolabo) in carbon tetrachloride

and air-dried—the solvent system being acetone–water, vaseline saturated (95:5, v/v); (b) thin-layer chromatography on silica gel impregnated with silver nitrate, the gel system being prepared using plates (Merck F_{254}) impregnated by soaking in a 25% solution of silver nitrate in acetonitrile[11] and air dried, while the solvent for development of the chromatogram was hexane–ethyl methyl ketone (8:2, v/v); (c) mass spectrometry*.

TABLE I

PAPER CHROMATOGRAPHY OF MENAQUINONE FROM DESULFOVIBRIO

(a) Reverse phase method (b) Silver nitrate impregnated thin-layer method.

Menaquinone	R_F	
	(*a*)	(*b*)
MK-5	0 59	0.38
MK-6	0 50	0.30
MK-7	0.38	0 22
Menaquinone (*D desulfuricans*)	0 49	0 31
Menaquinone (*D gigas*)	0 47	0 31

TABLE II

MASS SPECTRA OF MK-6 AND MENAQUINONES OF DESULFOVIBRIO

For interpretation of fragment structures see refs 12 and 14.

Fragmentation pattern (*m/e*)	% *of base peak*		
	MK-6 230°	*D desulfuricans menaquinone* 210°	*D gigas menaquinone* 210°
580	17	29	29
565	0 5	1	1
512	1 5	1 5	1.8
497–498	0 5	—	—
443–444	1 1	1 3	1 2
430	0 7	—	—
375–376	1 8	1 7	1 4
362	0 4	—	—
307–308	4 5	5	4
293–294	1 1	—	0 9
265	3.1	3	2 6
252	2	—	1.4
239	12	12	11
255	47	42	50
186	14	12	13
149	4 8	5 5	4 3
135–137	12	14	11
121–123	12	12	8 6
109	13	15	10
81	50	49	43
69	100	100	100

* Mass spectra were made with an A E I.MS-9 mass spectrometer with direct introduction into ionisation chamber (temperature, 210–230°; 70 eV), we thank the Mass Spectrometry Service of the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, and Prof. E Lederer, for their cooperation

The R_F values obtained in Methods (a) and (b) are given in Table I. The combination of these two methods assures that the quinone is menaquinone-6. The results of mass spectrometry (Table II) leave no doubt that this result is correct.

The fact that essentially only one menaquinone is present is reminiscent of similar findings in many photosynthetic bacteria which show only one quinone present—in these cases a benzoquinone (ubiquinone)[9]. Menaquinone-6, itself, is recorded but rarely in the literature, and then as a component in the electron transport systems of aerobic Micrococcus species[13].

The functional role of MK-6, as well as the implications of its presence in Desulfovibrio for bacterial evolution of electron transport systems, remain to be investigated.

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