MENAQUINONE-6 IN THE STRICT ANAEROBES DESULFOVIBRIO VULGARIS AND DESULFOVIBRIO GIGAS[1]

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Received November 4, 1969

Identical naphthoquinones have been isolated from the strict anaerobic sulfate reducing bacteria D. vulgaris and D. gigas. The vitamin was identified from both organisms as a menaquinone-6 (MK-6) by its characteristic chromatographic mobility and by mass spectral analysis. As the organisms possess cytochrome c_3 and carry out phosphorylation coupled to the oxidation of hydrogen with sulfite as electron acceptor, it is possible that MK-6 is involved as an oxidation-reduction component in the electron transport pathway in these anaerobes.

The sulfate reducing bacteria are strict anaerobic microorganisms that are capable of utilizing sulfate as a terminal electron acceptor (1). When molecular hydrogen, lactate, or ethanol function as electron donors for the reduction of sulfate to sulfide, the mechanisms of these mixed fermentations indicate that substrate phosphorylation cannot produce sufficient ATP to account for the growth of the bacteria (2). Phosphorylation coupled to the oxidation of hydrogen with sulfite as electron acceptor has been observed in extracts of one of these microorganisms, D. gigas. The phosphorylation was uncoupled by pentachlorophenol, gramicidin, and 2,4 dinitrophenol, required both soluble and particulate protein components, and appeared to be comparable to other such systems found in bacteria (3).

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[1] This work was supported by Grants AI-03046, AM-09909, and AM-19879 from the National Institutes of Health, United States Public Health Service.

These bacteria synthesize a single cytochrome, cytochrome c_3, which is required for electron transfer from hydrogen to enzymes presumably involved in the pathway of sulfate reduction by D. vulgaris (1). In addition, ferredoxin and flavodoxin have been implicated in the pathway of sulfate reduction in D. gigas (4, 5). Biochemical evidence pertaining to the possible relationship of the sulfate reducing bacteria to both the Thiorhodaceae and Clostridia has been summarized by several investigators (6, 7, 8). These speculations plus the presence of a cytochrome and oxidative phosphorylation during sulfate respiration have prompted a search for quinones in these organisms. In this paper, the isolation and identification of a naphthoquinone from two species of sulfate reducing bacteria are described.

EXPERIMENTAL RESULTS

Growth of Organisms

D. gigas was grown on a lactate-sulfate medium and the cells were harvested and washed as described by Le Gall, Mazza, and Dragoni (8). D. vulgaris (strain Hildenborough) was also grown on a lactate-sulfate medium (9), harvested and washed with 0.05 M phosphate buffer, pH 7.3. The purity of each culture was examined microscopically and any questionable cultures discarded. Washed cells of each organism were lyophilized and stored at -20° C.

Purification and Identification of the Naphthoquinone

The lyophilized organisms were extracted with acetone after suspension in a small amount of water. The extract was taken to dryness, the residue dissolved in hexane, and chromatographed on permutit using hexane with increasing concentrations of ether as the eluting solvent. Material with the absorption characteristics of vitamin K was eluted with 5% ether. Those

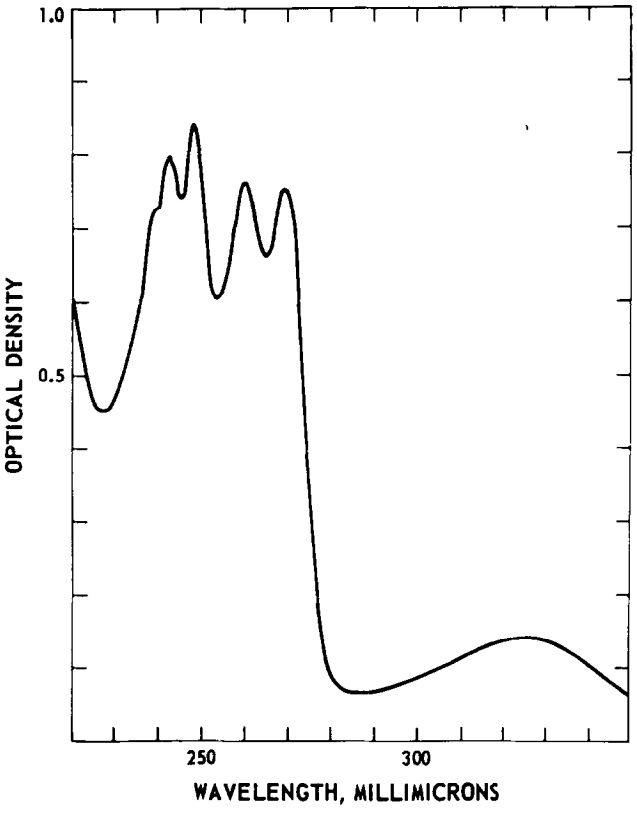

Fig. 1. Ultraviolet abosrption spectrum of naphthoquinone from D. vulgaris. The sample was dissolved in hexane and recording was made on a Cary Model 14 spectrophotometer.

fractions were chromatographed on silicic acid with increasing concentrations of benzene in hexane as eluting solvent (10) to give a fraction with the ultraviolet absorption spectrum shown in Fig. 1 (absorption maxima: 326, 269, 260, 248, 243, and a shoulder at 239 mμ). Extracts of both organisms showed identical spectra.

Chromatography of the naphthoquinone from each organism on thin layers of silica gel G impregnated with paraffin showed a principal spot corresponding to MK-6 (Fig. 2). The authentic samples of MK-4, 5, 6, and 9 were a gift of Dr. O. Isler. MK-7 was kindly provided by Dr. E. A. Doisy. Phylloquinone was purchased from Nutritional Biochemicals.

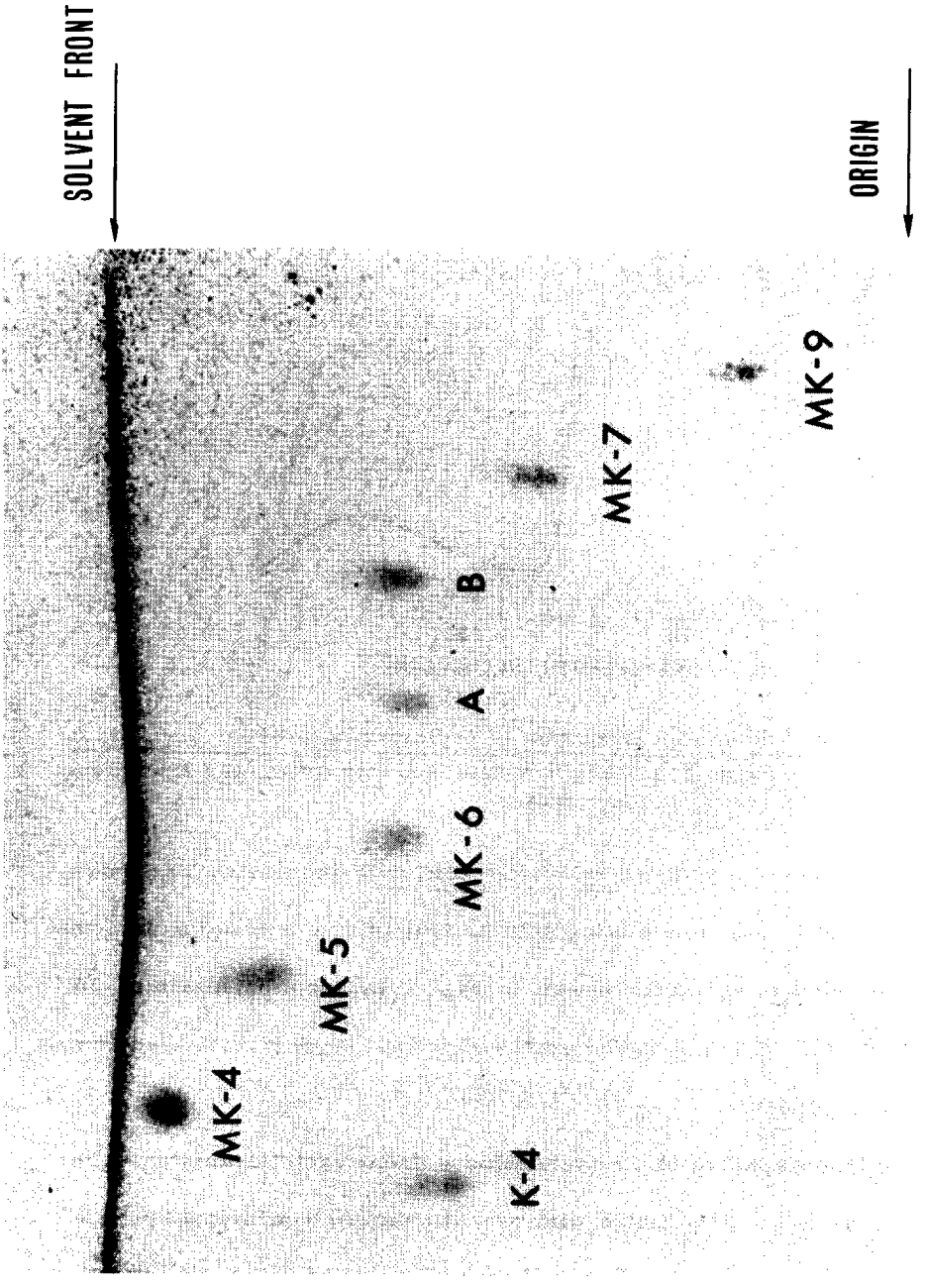

Fig. 2. Thin-layer chromatogram of reference compounds and the naphthoquinone isolated from D. vulgaris (A) and D. gigas (B). The specimens were spotted on silica gel G impregnated with paraffin and developed (ascending) with acetone-water 92:8. The plate was sprayed with concentrated H_2SO_4 and heated at 110° C for 15 min.

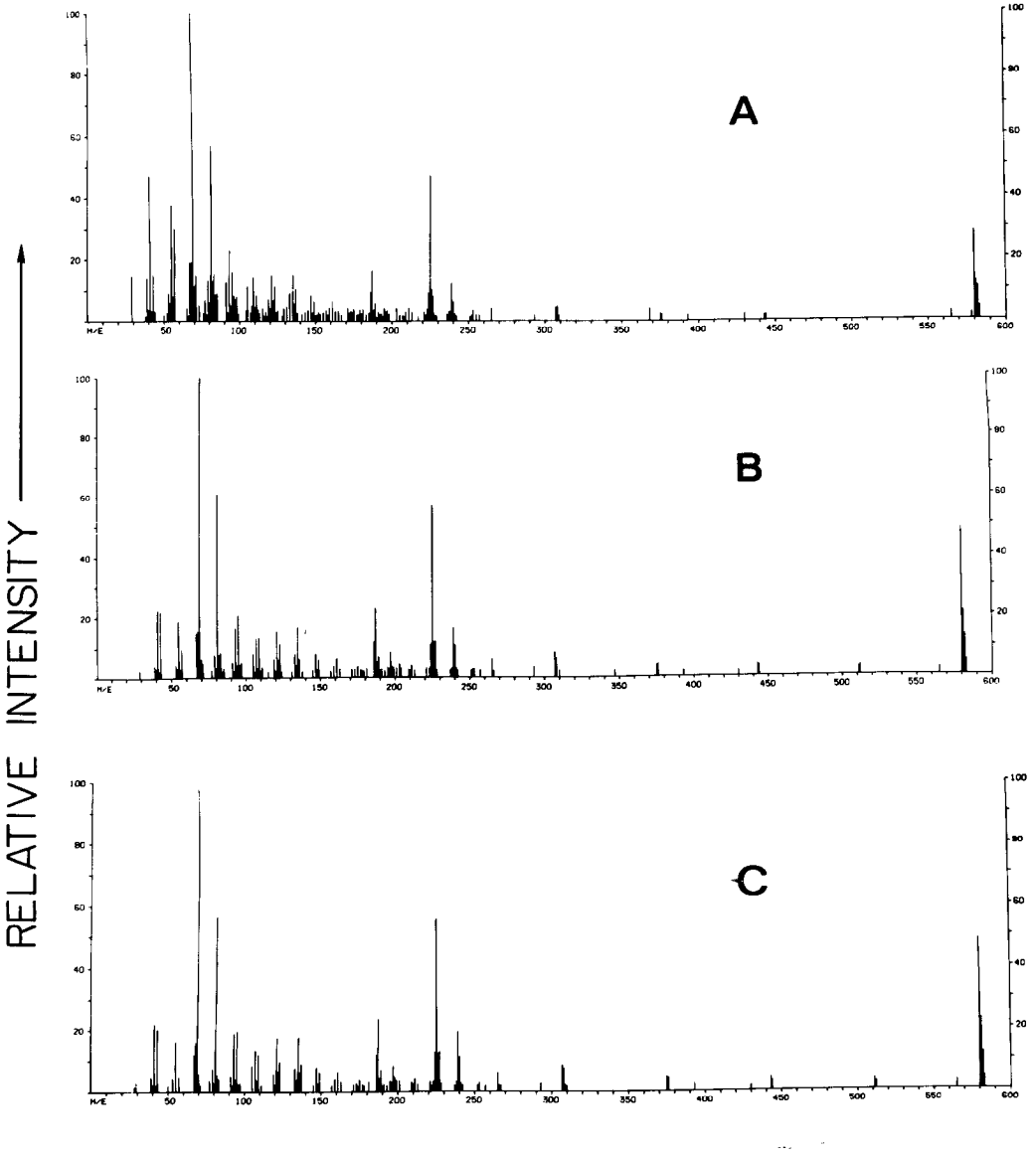

Fig. 3. Mass spectra of the naphthoquinones isolated from D. vulgaris (A) and D. gigas (B) and the synthetic MK-6 (C). For sample A, accelerating voltage 3.0 kv, ion source 70 ev, 310° C. For samples B and C, accelerating voltage 3.5 kv, ion source 54 ev, 280° C.

The structure of the isolated vitamins was confirmed by mass spectral analyses (LKB 9000 mass spectrometer fitted with a direct inlet probe).

As shown in Fig. 3, the naphthoquinones from both organisms gave the same spectra as synthetic MK-6 with an appropriate molecular ion at m/e 580 and the characteristic pyrylium ion at m/e 225 (11). The predominant spots corresponding to MK-6 in Fig. 2 and the molecular ion at m/e 580 shown in Fig. 3 preclude the presence of significant amounts of MK-6(2H) or other molecular species of the vitamin in either preparation.

DISCUSSION

Naphthoquinones have been shown to play a role in electron transport (12, 13) and oxidative phosphorylation (14) in bacteria. Bishop et al. (15) have found that aerobic bacteria contain either ubiquinone, menaquinone, or both. In some faculatative organisms they found that the menaquinone content did not vary in the presence or absence of oxygen, whereas in Staphylococcus albus there was a much lower content of menaquinone in cells grown anaerobically. Likewise, Kashket and Brodie (16) indicated that in E. coli both the naphthoquinone and ubiquinone content decreased under anaerobic conditions. However, Lester and Crane (17) found that E. coli grown anaerobically produced menaquinone but no ubiquinone. This was confirmed in a more recent study by Polglase et al. (18) who showed that menaquinone was present in only small amounts in E. coli grown aerobically, but increased in concentration under anaerobic conditions. This was in contrast to the ubiquinone content which was markedly reduced in concentration when the organisms were grown anaerobically.

In most strict anaerobes studied (15, 17), menaquinones and ubiquinones appear to be absent. However, Lev (19) isolated a strain of the anaerobe Fusiformis nigrescens which required a naphthoquinone for growth. Subsequently Gibbons and Engle (20) detected naphthoquinones in the naphthoqui-

none requiring strain of Bacteroides melaninogenicus (identical to F. nigrescens) and in a strain of B. fragilis and Veillonella alcalescens.

In the study reported here, two species of the strict anaerobes in the genus Desulfovibrio have been found to contain MK-6. As these organisms possess cytochrome c_3 and carry out phosphorylation coupled to the oxidation of hydrogen with sulfite as electron acceptor, the presence of MK-6 suggests that it may play a role in energy-linked reactions. Of interest is that these organisms are gram negative but did not appear to contain ubiquinone. This apparently is an exception to the results obtained by Bishop et al. (15), on a variety of organisms, that gram negative bacteria contain either ubiquinone and menaquinone or ubiquinone alone. The gram positive species studied contained only menaquinone.

The menaquinone identified in these studies has not been commonly found, but was identified earlier as trace component in putrified fish meal (21). The organism containing MK-6, however, was not identified. Greater amounts of MK-6 were detected more recently in several strains of aerobic micrococci (22).

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