Short Communication

A new structural type of methionaquinones isolated from hot spring sulfur-turf bacterial mats

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Bacterial respiratory quinones have great structural variations, depending on species and genera. To take advantage of this, the quinone profiles are used not only as chemotaxonomic criteria in the classification and identification of bacterial strains (Collins and Jones, 1981), but also as good measures of in situ bacterial biomass in terms of quantity, quality, and activity (Hiraishi, 1989). The major structural classes of bacterial quinones are menaquinones and ubiquinones with various numbers of isoprene units in their multiprenyl side chain. Besides these representatives, rare structural types of quinones, such as sulfur-containing quinones, occur in certain bacterial species. Methionaquinones (MTKs) represent one of the sulfur quinone groups, and they have so far been found in species of the genus *Hydrogenobacter* (Ishii et al., 1987; Kawasumi et al., 1984; Shima and Suzuki, 1993). During the study on community structure analysis of hot spring microbial mats by quinone profiling (Hiraishi et al., 1999), we found a new structural type of MTKs in so-called sulfur-turf bacterial mats (Maki, 1991; Miyoshi, 1897). This communication describes the characterization and identification of this new sulfur-turf quinone.

Hot spring sulfur-turf mats were collected from Ganiba (Akita Prefecture), Nakanoyu (Nagano Prefec-

ture), and Yumata (Nagano Prefecture), Japan. The samples were taken in sterile polyethylene bottles and transported in an insulated cooler to the laboratory. The biomass of the mats was harvested by centrifugation, washed with 50 mM phosphate buffer (pH 7.0) containing 1 mM ferricyanide, and stored at –20°C until analysis. Washed samples (ca. 3 g wet wt) were resuspended in this buffer, mixed with 2.5 volumes of a chloroform-methanol mixture (2:1, v/v), and sonicated on ice for 2 min with 2 s intermittent bursts (20 kHz; output power, 100 W). The homogenates were then centrifuged at 5,000×*g* for 10 min. The resultant upper aqueous layer was discarded, and the lipid layer was collected by a pipette. Every 30 min the residue was extracted once with acetone and then twice with the chloroform-methanol mixture. All extracts were combined, evaporated under a vacuum, and reextracted three times with *n*-hexane-1% saline (1:1, v/v). The hexane extract was concentrated and applied to the top of a Sep-Pak Vac cartridge (Waters Corp., Milford, MA), which had been washed with acetone and equilibrated with *n*-hexane. Quinones in the column were eluted with 15 ml of 1% diethyl ether-hexane, then with 15 ml of 10% diethyl ether, to obtain menaquinone and ubiquinone fractions, respectively (Hiraishi et al., 1996). The extracts were concentrated and dissolved in 100 µl acetone for the analysis. Quinone components were analyzed and identified by reverse-phase HPLC with an ODS column (4.6×

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Fig. 1. HPLC elution patterns of the quinones from the Nakanoyu sulfur-turf mat (a) and *Hydrogenobacter thermophilus* JCM 7678 (b).

Ultraviolet absorption spectra of the main components are shown on the right side. Quinones were eluted with methanol-isopropyl ether (9:2, v/v) as the mobile phase at a flow rate of 1 ml/min.

250 mm) and photodiode array detection, mass spectrometry, and silver-ion TLC, as previously described (Hiraishi et al., 1992, 1996). Standard menaquinones and ubiquinones were prepared from raw sewage and activated sludge, as previously reported (Hiraishi et al., 1996). An MTK standard, MTK-7(H_4) (2-methylthio-3-VI, VII-tetrahydromultiprenyl⁷-1,4-naphthoquinone), was prepared from cells of *Hydrogenobacter thermophilus* JCM 7687.

The major quinone components from all sulfur-turf mats were contained in the fraction eluted with 1% diethyl ether-hexane with a Sep-Pak Vac cartridge. The eluents with 10% diethyl ether-hexane contained only trace amounts of quinones, most of which were represented by ubiquinone-8. HPLC experiments revealed that the 1% diethyl ether fractions from all sulfur-turf samples produced a main quinone component that had an HPLC retention time of 13.0 min (Fig. 1a) and that showed absorption maxima at 239–240, 261 (maximum), and 312–317 nm (Fig. 1a, right). This component accounted for 65 to 95% (as HPLC peak area) of the total quinone contents of the sulfur-turf

Fig. 2. Mass spectra of the HPLC-separated MTK components from the sulfur-turf mat (a) and *Hydrogenobacter thermophilus* JCM 7678 (b).

mats (data not shown). The spectral pattern of the major sulfur-turf quinones was the same as that of MTK-7(H_4) purified from *H. thermophilus* JCM 7687 (Fig. 1b, right). However, the main component of the sulfur-turf mats differed in HPLC elution time from the *H. thermophilus* MTK, which was eluted at 17.9 min. This suggested that the sulfur-turf quinones were an MTK homolog differing from MTK-7(H_4) in the structure of the multiprenyl side chain.

To ascertain the chemical structure of the main sulfur-turf quinones, mass spectroscopy and silver-ion TLC were performed. The possible MTK component from the sulfur-turf samples had a molecular ion peak ($M+1$) at m/z 681.6 (Fig. 2a). This value is 4 mass units lower than the value for *H. thermophilus* MTK-7(H_4) (Fig. 2b). Furthermore, a mass peak of $M+1-46$ (m/z 635.6), which resulted probably from the elimination of the fragment $S=CH_2$, was observed in the sulfur-turf MTKs, like the case of MTK-7(H_4) (Ishii et al., 1987). Silver-ion TLC showed that the sulfur-turf MTKs moved much faster than the *H. thermophilus* MTK and migrated similarly with unsaturated menaquinones at a front of the developing solvent (data not shown). This suggested that no part of the multiprenyl side chain of the sulfur-turf MTKs is saturated with hydrogen atoms, unlike the *H. thermophilus* MTK.

Fig. 3. Chemical structures of a new methionaquinone (MTK-7) from the sulfur-turf mats (a) and of *Hydrogenobacter thermophilus* MTK (b).

Thus because of the results of spectrochromatography, mass spectrometry, and silver-ion chromatography, the major MTK components of the sulfur-turf mats were identified as fully unsaturated MTK-7 (2-methylthio-3-multiprenyl⁷-1,4-naphthoquinone) (Fig. 3).

Methionaquinones have hitherto been found as the sole quinone in the thermophilic chemolithotrophic bacteria, *Hydrogenobacter* species, and MTK-7(H₄) is the major homolog in all these bacteria (Ishii et al., 1987; Kawasumi et al., 1984; Shima and Suzuki, 1993). Therefore our report is the first demonstration of the natural occurrence of an MTK homolog other than MTK-7(H₄). The predominant bacteria of the sulfur-turf mats have not yet been isolated as culturable strains, but a culture-independent molecular approach has shown that they represent a deeply branching line of descent within the *Aquifex-Hydrogenobacter* phylum (Yamamoto et al., 1998). The results of this study strongly suggest that the sulfur-turf bacteria of the above-noted phylotype contain MTK-7 as the major quinone component, and this chemotaxonomic feature is consistent with their phylogenetic positions as members of the *Aquifex-Hydrogenobacter* complex. Although no information has been made available on quinone systems of members of *Aquificales* except for *Hydrogenobacter* and the sulfur-turf bacteria, MTKs are suggested to be essential components of the respiratory chains in this earliest branching phylogenetic group of the domain *Bacteria* (Pitulle et al., 1994).

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