Atmospheric pressure chemical ionization and atmospheric pressure photoionization for simultaneous mass spectrometric analysis of microbial respiratory ubiquinones and menaquinones

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An atmospheric pressure photoionization (APPI) source and an atmospheric pressure chemical ionization (APCI) source were compared for the selective detection of microbial respiratory ubiquinone and menaquinone isoprenologues using tandem mass spectrometry. Ionization source- and compound mass-dependent parameters were optimized individually for both sources, using the available quinone standards. Detection levels for the two ion sources were determined with ubiquinone-6 (UQ6) and menaquinone-4 (MK4, vitamin K2) standards using flow injection analysis and selected reaction monitoring (SRM). With APPI the calculated lower limit of detection (LLOD) was 1.7 fmol µl⁻¹ for UQ6 and 2.2 fmol µl⁻¹ for MK4 at a signal-to-noise ratio of 3. These LLODs were at least three times lower than with APCI. The selectivity of detection afforded by SRM detection reduced complex mixture analysis to 3 min per sample by eliminating the need for chromatographic separations. The detection method was successfully applied to quinone quantification in a variety of environmental samples and cell cultures. Adequate amounts of respiratory quinones can be extracted and quantified from samples containing as low as 2 × 10⁷ cells. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: atmospheric pressure photoionization; atmospheric pressure chemical ionization; ubiquinone; menaquinone; tandem mass spectrometry

INTRODUCTION

The ability of some microbes to adapt to the diverse conditions of their environment is a direct result of their capacity to form different respiratory quinones in response to the presence of different terminal electron acceptors. Aerobic growth with oxygen as the terminal electron acceptor induces Gram-negative bacteria and eukaryotes to form ubiquinones (UQₙ, 1-methyl-2-isoprenyl-3,4-dimethoxyparabenzoquinone) with the 2-isoprenyl chain containing 1–14 isoprene (n) units. Gram-negative bacteria when grown anaerobically, and also many other bacteria, form menaquinones (MKₙ, 1-isoprenyl-2-methyl-napthoquinone). Some organisms form demethylated MKₙ (DMKₙ) if grown under anaerobic conditions with an electron acceptor such as nitrate. The UQₙ/MKₙ ratio of microbial communities has proved to be an excellent measure of the terminal electron acceptor concentrations and fluxes that integrates over time. In addition, the quinone isoprenologue profile provides insight into the composition of microbial communities. Therefore, the respiratory quinone profiles of microorganisms are useful biomarkers to characterize both the community composition and, for Gram-negative bacteria, the in situ microniche redox environment. In studies of bioremediation, insights into the redox environments of the microbiota can be of vital importance in determining the success of the cleanup processes.

In previous studies, utilization of UQₙ/MKₙ ratios as a measure of terminal electron acceptor flux has required serial
ex Extractions of large sample sizes and subsequent purification by thin-layer chromatography, because the concentrations per cell are low. For example, UQ, 10 dry weight and MK, about 0.02–0.1 µmol g⁻¹ dry weight in an organism such as E. coli, in contrast to the phospholipids, which are present at about 100–150 µmol g⁻¹ dry weight. Quinone detection by single quadrupole mass spectrometry (MS) with a frit-fast atom bombardment (FAB) source has shown greater sensitivity than diode-array detection, but still requires 1–10 mg of dry cells. The utility of electrospray tandem mass spectrometry (ES-MS/MS) in quantifying UQ from ammonium acetate addition via a pre-column. Formation of the ammonium ion adduct of the ubiquinones, which are present at about 100–150 µmol g⁻¹ dry weight.

Quinone detection by single quadrupole mass spectrometry (MS) with a frit-fast atom bombardment (FAB) source has shown greater sensitivity than diode-array detection, but still requires 1–10 mg of dry cells. The utility of electrospray tandem mass spectrometry (ES-MS/MS) in quantifying UQ from ammonium acetate addition via a pre-column. This allowed direct UQ analysis in the neutral lipid extracts from several environmental samples, sometimes via the Gram-negative microbial community. Unfortunately, in our hands, MK and DMK were not detectable by ES-MS/MS. Tests for ionization of menaquinones using the electrospray source did not yield sufficient amounts of molecular ions, fragment ions, or ammonium adduct ions (data not shown), as also mentioned by Lytle et al. Therefore, we tested other ionization sources for efficient ionization of menaquinones and ubiquinones. Here we show that the molar ratio MK/UQ, an extremely valuable biotechnological parameter, can be directly determined on neutral lipid samples from microbial communities, using atmospheric pressure chemical ionization (APCI)-MS/MS or atmospheric pressure photoionization (APPI)-MS/MS in the selected reaction monitoring (SRM) mode. Both types of quinones were readily ionized and detected with either ion source, and hitherto unrivalled small amounts of microbial cells (sample material) were needed to obtain UQ/MK ratios, and profiles. However, APPI proved to be at least a factor of three times more sensitive than the APCI source, which is important for the detection of the small amounts of ubiquinones and menaquinones extractable from environmental samples.

**EXPERIMENTAL**

**General MS**

All experiments were carried out with an APCI or a prototype APPI source connected to an API 365 triple-quadrupole mass spectrometer (MDS SCIEX, Concord, Ontario, Canada) operated in the positive mode. The same prototype APPI source connected to an API 365 triple-quadrupole mass spectrometer (MDS SCIEX, Concord, Ontario, Canada) operated in the positive mode. The same prototype APPI source connected to an API 365 triple-quadrupole mass spectrometer (MDS SCIEX, Concord, Ontario, Canada) operated in the positive mode.

**High-performance liquid chromatography (HPLC)**

An Agilent 1100 LC system was used for flow injection analysis (FIA) and for HPLC of quinones. Flow-rates were tested with the APCI and the APPI source ranging from 50 to 200 µl min⁻¹ of methanol–chloroform (80:20, v/v) for FIA. Methanol or methanol–2-propanol (80:20, v/v) at 100 µl min⁻¹ was used for chromatographic separation of quinones on HAISIL 300 x 1 mm i.d. (Higgins Analytical by Chromtech, USA) and Hypersil RP-18 (ODS) 150 x 1 mm i.d. (3 µm) (Phenomenex, Torrance, CA, USA) microbore LC columns.

**Quinones**

The nomenclature for quinones designates the respective core structure, indicated by the number of units in the isoprenyl side-chain. Ubiquinone standards UQ, UQ, UQ, UQ, and the menaquinone MK (vitamin K), were obtained from Sigma (St. Louis MO, USA) and used as received. The ubiquinone UQ was isolated in triplicate from 5 mg of E. coli lyophilized cells (Sigma) as described below. To obtain product ion scans, determine diagnostic ions and optimize source-dependent parameters the standards were dissolved in methanol–chloroform (80:20, v/v) and infused, through a tee into an additional solvent stream, at 10 µl min⁻¹ using a syringe pump. The major solvent stream was provided by an Agilent 1100 quaternary pump to obtain a final flow-rate of 100–200 µl min⁻¹ recommended for APCI and APPI, and suitable for the dimensions of the microbore LC columns used.

**Flow cell biofilm**

Acidovorax delafieldii 670a, Bacillus sp 3b47e and P. putida sp were grown and then enriched in a biofilm accumulation chamber (BAC) using a constant stream of liquid delivering oxygen and nutrients as described previously. To the biofilms grown for 48 h, E. coli cells were added at concentrations from 10⁷ to 10⁸ cells to simulate an environmental stress factor, and the supply of nutrients and oxygen was stopped to one out of four BACs. After 24 h the biofilms from the BACs were extracted and the neutral lipid fraction obtained by silicic acid column chromatography (see below).

**Cell cultures**

Enterococcus faecalis (ATCC 11420) cells were grown in ATCC’s 125 TYG medium under anaerobic conditions. After 24 h, 100 ml of culture medium (3.0 x 10⁶ cells ml⁻¹) were centrifuged, the supernatant was removed and then 10 ml of one-phase extraction solvent were added to the cell pellet. Wolinella succinogenes and Geobacter sulfurreducens cell material was lyophilized in ammonium acetate buffer (pH 6.5, B. Schink, Konstanz, Germany) and 15 mg cell dry weight suspended in 10 ml of extraction solvent.

**Sample preparation**

Quinones were extracted from frozen samples or lyophilized cell material (E. coli) by a one-phase modified Bligh/Dyer extraction method. The organic phase extracts were dried under a gentle nitrogen stream. Residues were resolved in 100 µl of chloroform and fractionated on 500 mg of activated
RESULTS AND DISCUSSION

Optimization of APCI

For the compounds under study, the major ions observed with APCI were the protonated molecules, [M + H]^+ . For ubiquinone-6 (UQ₆), the base peak in the spectrum of a Q1 scan was [M + H]^+ at m/z 591.7. The product ion spectrum of m/z 591.7 and of the other ubiquinone protonated molecules investigated showed a base peak at m/z 197 (Fig. 1). We assume that this fragment ion is the benzylium ion as it is a resonance-stabilized structure, and was identified previously by ES-MS/MS studies of these compounds.⁸

The mass spectrum of MK₄ (vitamin K₄) showed the protonated molecule [M + H]^+ at m/z 445. The major ion in the product ion spectrum of m/z 445 was detected at m/z 187, which represents the 2-methylnapthoquinone core. All other menaquinones studied fragmented in a similar manner, producing this diagnostic ion (Fig. 1).

The SRM transitions used for the detection of 11 major UQs and MKs are listed in Table 1. Mass analyzer parameters were optimized individually for each of the available quinone standards to yield maximum signal intensity in the positive mode with the APCI source. For quinones without authentic standards available, optimum mass-dependent parameters such as voltages on orifice, collision energy and cell entrance potential were calculated assuming a linear change with increasing number of isoprene units in the side-chain. The collision energy (CE) needed to be increased with increasing mass of the parent quinones to maintain approximately the same center-of-mass collision energy, viz. 1.6 eV for UQ₆ and 1.8 eV for MK₄. Optimum conditions were further verified and adjusted using quinone extracts of cell cultures from microorganisms with known quinone composition. For example, the parameters for UQ₈ were obtained using the neutral lipid fraction from E. coli (see sample preparation), as UQ₈ represented 90% of the total quinone content of cells grown under aerobic conditions.⁵ MK₄ was used for initial optimization of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Q1 (u)</th>
<th>Q3 (u)</th>
<th>DPʰ</th>
<th>FPᶜ</th>
<th>CEPᵈ</th>
<th>CEᵉ</th>
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<tr>
<td>UQ₆</td>
<td>591.5</td>
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<td>210</td>
<td>20.5</td>
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<td>190</td>
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<td>175</td>
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<td>MK₈</td>
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<td>187.0</td>
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<tr>
<td>MK₉</td>
<td>786.1</td>
<td>187.0</td>
<td>30</td>
<td>210</td>
<td>31.4</td>
<td>52</td>
</tr>
</tbody>
</table>

¹ The parameters were optimized using dilution of standards or using the purified neutral lipid fraction of lipid extracts from microorganisms with known quinone content. The scan time was 250 ms for each mass transition.
² Voltage between orifice and skimmer (ground).
³ Voltage between skimmer (ground) and ring potential.
⁴ Collision cell entrance potential.
⁵ Collision energy for collision-induced dissociation.

Figure 1. General structure of ubiquinones and menaquinones shown for UQ₆ and MK₄. The product ion spectra for m/z 591.7 (UQ₆) and m/z 445.5 (MK₄) revealed fragment ions, indicated with their m/z and structure, characteristic for the respective quinone class. The molecular ion [M + H]^+ and the respective diagnostic fragment ion, major ion in the mass spectra, were used for SRM.
detected parameters as it was the only menaquinone standard available. In a second step we obtained neutral lipid extracts of anaerobically grown *Wolinella succinogenes* and *Geobacter sulfurreducens* cultures (a gift from B. Schink, University of Konstanz, Germany), and determined the menaquinones in the extracts by monitoring the parent ions of *m/z* 187. For the *Wolinella* strain we found *m/z* 581 as the major parent ion, which matches the calculated mass for [M + H]+ of MK6. The *Geobacter* strain contained mainly *m/z* 718 and minor amounts of *m/z* 786 (10%), corresponding to [M + H]+ of MK5 and [M + H]+ of MK6 respectively. The settings for menaquinone detection were individually optimized for the following mass transitions in SRM: *m/z* 581/187, 718/187 and 786/187 and the values for *m/z* 513/187 (MK5) and *m/z* 650/187 (MK6) extrapolated afterwards (Table 1).

**Optimization of APPI**

The APPI source is based on the same heated nebulizer probe used for APCI and therefore most of the settings were used as optimized with the APCI source. However, the heated nebulizer at the APPI source has a spray voltage instead of the nebulizer current. The optimum APPI spray voltage was determined with UQ6 and MK4 using SRM and either acetone or toluene as the dopant. Signal levels were maximized at spray voltages of 1.3–1.7 kV with toluene as the dopant as recommended in the preliminary APPI source manual. A source gas temperature of APPI of ~325°C yielded a slightly higher relative signal intensity for UQ6 and for MK4 (15 ± 5%) compared with the 425°C determined as the optimum APPI (not shown). An expected gain in detection sensitivity using APPI at lower temperatures can be compromised owing to sample carryover and peak tailing, therefore 425°C was used in all further experiments for comparison of quinone quantification between the two ion sources.

With both APPI and APCI, the signal intensity at equal concentrations was always lower for MK4 than for UQ6, even after individual parameter optimization for the respective mass transitions. This is attributed to a lower stability of the napthoquinone core, which is indicated by the following findings. First, in a Q1 scan (*m/z* 400–1000 u), with infusion of MK4, an increase in the declustering potential increases the abundance of a fragment ion [M − 14]+, which corresponds to an loss of the 2-methyl from the menaquinone core in the source ionization region. Second, the relative abundance of low-mass fragment ions other than *m/z* 187 (>30% for *m/z* 109, 149 and 213) was significantly higher than observed for low-mass fragment ions of UQ6 (<10%; Fig. 1). Decreasing the collision energy for collision-induced dissociation (CID) did not change the signal intensity of low-mass ions relative to the fragment ion at *m/z* 187 (data not shown). Both findings cause a decrease in signal intensity in the SRM of [M + H]+/*m/z* 187. The structure and formation pathways of the menaquinone low mass fragment ions are unknown.

**Detection Limits**

Calibration curves for the quinones MK4 and UQ6 showed a linear response in the range from <3 to 8999 and 6670 fmol μL−1, respectively, for APPI. The range of linear response started at slightly higher quinone concentrations of 9.0 and 6.7 fmol μL−1, respectively, for APCI. The correlations (r values) for all available quinone standard calibrations were r > 0.9987 and r > 0.9957 with APPI and APCI, respectively. The signal intensity standard deviations were <10% with APPI and <15% with APCI for the whole concentration range. Using FIA, the respective lower limit of quantification (LLOQ) with APPI and APCI at a signal-to-noise ratio (S/N) >9 was determined as 2.8 and 9.0 fmol μL−1 of MK4, respectively. Compared with MK4, the LLOQ for UQ6 was slightly better with both ion sources (2.1 and 6.8 fmol μL−1, respectively). The quantification of UQ10 was less sensitive than that of UQ6, as nearly double the amount was needed with APPI (4.6 fmol μL−1), and four times with APCI (23.2 fmol μL−1) to obtain an S/N >9 (Fig. 3). Overall,
the APPI source proved to have better detection levels (at least a factor of three times better) than the APCI source, and also smaller standard deviations (in replicate analysis) for all quinones tested.

**Separation of quinones**

Partial separation of quinone isoprenologues can be achieved on a short microbore RP-18 column at low flow-rates, according to our existing method for the detection of ubiquinones by ES-MS/MS with the pre-column addition of 10 mM ammonium acetate as solvent modifier. However, the ammoniated ion at $m/z$ [M + NH$_4$]$^+$ known from ES of ubiquinones was not produced in the corona discharge gas phase according to an obtained Q1 mass spectrum of UQ$_6$ (APCI-MS data not shown). In the SRM mode (see Table 1), the signal intensities were comparable for a standard mix containing UQ$_6$, UQ$_7$, UQ$_9$, and UQ$_{10}$, whether 10 mM ammonium acetate was added or not, whereas addition of 20 mM ammonium caused a significant decrease in the signal intensities (Table 2). At the latter concentration of ammonium, the ionization of these molecules may be significantly favored over ionization of the ubiquinones. As ammonium acetate did not improve the ionization in either APCI or APPI, it can be omitted.

Whether ammonium acetate was added or not, the retention times of ubiquinones increased with their isoprenoid chain length (UQ$_6$ to UQ$_{10}$), and MK$_4$ eluted very shortly before UQ$_6$, indicating that menaquinones have a higher retention time than the respective ubiquinones (Fig. 4). The differences in retention time between the quinone peaks decreased significantly if the flow-rate was increased for APCI to 100 µl min$^{-1}$, e.g. 0.5 min elution time between the peak apex of UQ$_6$ and UQ$_{10}$, causing significant peak overlap (Fig. 4(A)). However, with SRM the individual quinones were easily resolved according to their unique mass transitions. Using 100% methanol as the solvent increased the retention time differences, but a complete baseline separation for all peaks could not be achieved on the short column (data not shown). With a longer column (Hypersil RP-18 (ODS), 150 × 1 mm i.d.) a baseline separation of ubiquinones and even of MK$_4$ and UQ$_6$ was possible with methanol–2-propanol (80:20) as solvent (Fig. 4(B)). However, significant peak broadening occurred, which compromises the detection sensitivity for ubiquinones with more than seven isoprenoid units and even later eluting respective menaquinones (not shown).

In any case, the relative abundances of quinones in mixtures separated by HPLC or simply determined by FIA were identical for quinone concentrations of 480 ppb. These data indicate that HPLC separation and the associated slower speed of analysis can be eliminated in favor of analysis by FIA with SRM detection. Apparently the ionization efficiencies among the various quinones at these levels are not compromised by simultaneous introduction into the ion source. Chromatographic separation of different quinones has been utilized in all currently established methods using an electrochemical electrode, diode-array detector or single-quadrupole mass spectrometer as detection devices. All these methods have the disadvantages of long analysis times, peak broadening for larger quinones and omitted detection of small menaquinones (MK$_4$ and MK$_5$).

To take advantage of the speed of analysis, FIA with SRM detection was employed in all subsequent analysis. Using isotopically labeled quinones as internal standards would provide the most exact method for quantification with MS/MS detection, but these were not commercially available. To obtain quantitative measurements of the

Table 2. Calculated concentration of quinone standard mix analyzed with APCI using 10 and 20 mM ammonium acetate as solvent modifier and without modifier

<table>
<thead>
<tr>
<th>Modifier</th>
<th>UQ$_6$</th>
<th>UQ$_7$</th>
<th>UQ$_9$</th>
<th>UQ$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM NH$_4$ acetate</td>
<td>7.8</td>
<td>4.8</td>
<td>24.3</td>
<td>47.8</td>
</tr>
<tr>
<td>20 mM NH$_4$ acetate</td>
<td>6.2</td>
<td>3.7</td>
<td>17.9</td>
<td>29.9</td>
</tr>
<tr>
<td>None$^a$</td>
<td>8.2</td>
<td>5.6</td>
<td>25.1</td>
<td>47.6</td>
</tr>
</tbody>
</table>

$^a$ Reference: values according to the respective standard concentration.

Figure 4. Separation of authentic quinone standards on an RP-18 HPLC column (A) in 3 min on HAISIL 300, 30 × 1 mm i.d. and (B) in 20 min on Hypersil RP-18 (ODS), 150 × 1 mm i.d. (100 µl min$^{-1}$, methanol–2-propanol (80:20)) detected with APCI-MS/MS. The signal intensities are relative to the highest peak (set to 100%) within the respective separation.
amount of quinones present in a mixture, an external standardization with a representative of each available quinone class was used. A stock solution of the quinones UQ₉, UQ₁₀, and MK₄ were prepared at four concentrations (e.g. UQ₉ at 1.7, 6.8, 17.0, and 170.0 fmol µl⁻¹) and aliquots measured before each sample set. Although quinones are known to be photosensitive and to disintegrate if exposed to oxygen, with storage at 4°C in MeOH and protection from light, measured concentration of standards showed no loss over a period of 6 months. This was verified by comparison of stored standards with freshly prepared solutions (data not shown).

### Analysis of environmental samples

One of the most useful environmental parameters is the molar ratio of ubiquinones to menaquinones (UQₙ/MKₙ). The absolute concentration in cells or other samples is less important. The simultaneous measurement of ubiquinones and menaquinones provides the best indication for relative quinone amounts within different samples. Peak area values for ubiquinones were normalized to UQ₆ standards and for menaquinones to MK₇ standards of known concentration (pg µl⁻¹) analyzed within the same sample sequence. Data were than converted to pmol values to obtain UQₙ/MKₙ ratios based on the molar concentrations in the samples.

We quantified complex quinone compositions in environmental samples successfully using detection of 11 SRM transitions without a column separation. The chosen mass transitions embraced the ubiquinones and menaquinones directly accessible for quantification as available standards, or indirectly accessible owing to extrapolation based on the respective comparable standards. With FIA we could detect almost all of these monitored quinones in a single sample (Fig. 5), containing a calculated total quinone content of 81 pmol. This corresponds to 2.7 x 10⁸ cells, or 1 nmol in 3.0 x 10⁶ cells. Based on profiles obtained from environmental samples and the determined detection limits, we propose suitability of the method for samples containing as low as 2.0 x 10⁶ cells, or approximately two orders of magnitude less sample material than necessary for quantification based on diode-array detection.

Menaquinones were induced in a Gram-negative bacterial community in microbial biofilm accumulation chambers with interruption of the supply of oxygen and nutrients, driving the environment anaerobic after addition of 10³ E. coli cells. The relative amount of MK₈ and MK₇ increased to 50 and 23%, respectively, within 24 h. If chambers were kept aerobic, addition of E. coli (10², 10⁶, 10⁸ cells) had no significant influence on the relative quinone distribution, clearly dominated by 80% UQ₆. The calculated molar ratio UQ₉/MK₉ changed from 6.1 (±0.61) for aerobic conditions to 0.3 for anaerobic conditions (Table 3). These ratios were detected in biofilms containing ~10⁸–10⁹ cells cm⁻² with the methods described here. We found comparable UQ₉/MK₉ ratios published for 4 l batches of E. coli liquid cultures, where the ratio of MK₉ to UQ₉ (major quinones in E. coli) was 17.3 at the log phase with vigorous aeration, and 0.01 under consequent anaerobic growth conditions, but nearly 1.0 in the aerobic stationary phase.

If an aerobic culture (in artificial groundwater) is rendered anaerobic, menaquinones are rapidly produced, whereas the ubiquinones produced with aeration survive at least 2 weeks in an anaerobic environment. Ubiquinones are not known to be produced by Gram-negative facultative aerobic microorganisms under anoxic conditions. This could possibly be reflecting the high redox potential of the ubiquinone-quinol system utilizing oxygen as a terminal electron acceptor. Therefore, we propose that quinone ratios indicate the redox state for a short-term history (weeks) of oxygen availability in the presence of Gram-negative aerobic, facultative aerobic or anaerobic bacteria. This hypothesis has been tested in vitro in reconstruction experiments. The UQ/MK ratio near zero indicates long-term (months) exposure to strictly anaerobic conditions. Values below 1.0 indicate anaerobic conditions with a history of past oxygen deprivation.

### Table 3. Distribution of quinones detectable in a constructed tri-culture biofilm 24 h after addition of E. coli (10²−10⁸ cells) and aerobic growth followed by switch to anaerobic growth (10⁴ E. coli cells added) (the respective ubiquinone to menaquinone ratio (UQₙ/MKₙ) is also shown)

<table>
<thead>
<tr>
<th>Flow cell</th>
<th>UQ₆</th>
<th>UQ₇</th>
<th>UQ₈</th>
<th>UQ₉</th>
<th>UQ₁₀</th>
<th>MK₄</th>
<th>MK₇</th>
<th>MK₈</th>
<th>Ratio UQ₉/MK₉</th>
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<tbody>
<tr>
<td>Aerobic + 10² cells</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
<td>81.0</td>
<td>2.4</td>
<td>2.1</td>
<td>8.4</td>
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<td>5.5</td>
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<tr>
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<td>0.8</td>
<td>1.8</td>
<td>83.0</td>
<td>2.2</td>
<td>1.5</td>
<td>8.5</td>
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<td>0.9</td>
<td>3.8</td>
<td>79.0</td>
<td>2.3</td>
<td>1.5</td>
<td>8.5</td>
<td>4.5</td>
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<td>50.0</td>
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</table>
availability, whereas values around 1.0 and above indicate an aerobic or partial microaerophilic environment. A low UQ/MK ratio can also be an indicator for archaea as they produce menaquinones. The presence of archaea would be reflected in a high MK/PLFA ratio.

Several respiratory quinones other than the major ubiquinones and menaquinones analyzed here have been reported in bacteria and archaea. Menaquinones showing single or multiple hydrogenations in the side-chain, expressed as MK\textsubscript{m}(H\textsubscript{m}), are common in actinomycetes and known from analysis of sewage activated sludge.\textsuperscript{4} Menaquinones with more than 10 isoprenoid units occur in Bacteroides spp.\textsuperscript{16} and ubiquinones with 12–14 isoprenoid units in the side-chain (UQ\textsubscript{12–14}) occur in Legionella spp.\textsuperscript{1} Additionally, isoprenoid quinone structures containing sulfur, nitrogen, additional methyl groups in the core, heterocyclic cores and/or modifications in the side-chain (e.g. epoxy, ketone) or a loss of a methyl from the menaquinone core (demethylmenaquinones, DMKs) have also been described. Sensitive quantification of these specific quinones would be useful in taxonomic determinations and analysis of microbial systems characterized by elevated temperatures, sufficient light for photosynthesis, conditions suitable for methanotrophs or anaerobic growth with nitrate as electron acceptor.\textsuperscript{16} The analysis of plastoquinones and phyloquinone will be important for aquatic environments.\textsuperscript{17} APPI- or APCI-MS/MS can simplify the search for specific quinones in cell cultures or unknown samples.

DMethylenamenaquinone-9 (DMK\textsubscript{9}) was detected in neutral lipid extracts of Enterococcus faecalis by a precursor ion scan for \textit{m/z} 173 (\textit{m/z} [187–CH\textsubscript{2}]\textsuperscript{+}), showing the expected peak for the protonated molecule \([\text{M}+\text{H}]^+\) at \textit{m/z} 772. We could also detect thermoplasmaquinone\textsuperscript{16} as a minor compound in a Wolinella succinogenes isolate with a similar approach using the fragment ion at \textit{m/z} 201 as diagnostic ion (not shown).

CONCLUSIONS

The results presented in this paper show that both APCI- and APPI-MS/MS making use of SRM detection can be used for the selective, low-level and quantitative detection of both ubiquinones and menaquinones. These capabilities allow the determination of the environmentally diagnostic UQ\textsubscript{m}/MK\textsubscript{m} ratio. With APPI-MS/MS, the determined lower limit of detection (LL LOD) was 1.7 fmol μL\textsuperscript{-1} for UQ\textsubscript{m} and of 2.2 fmol μL\textsuperscript{-1} for MK\textsubscript{m}. The detection levels determined with APPI-MS/MS were at least three times better than with APCI-MS/MS. Therefore, APPI-MS/MS would be the most suitable method for the detection and quantification of the low levels of respiratory quinone isoprenologues in environmental samples. However, even the quantification of respiratory quinones with APCI-MS/MS is a promising tool. Owing to the high selectivity of quinone detection using the SRM mode, sensitive quantification was possible if either LC with baseline separation, LC with partial peak separation or simple FIA was used. The highest signal intensities were obtained with FIA and partial peak separation on a short column, whereas on a longer column peak broadening compromised the detection sensitivity.

Using the here described APPI- or APCI-MS/MS approach for specific and sensitive detection, and quantification of the major isoprenoid ubiquinones and menaquinones, a wide variety of structurally modified respiratory quinones should be detectable with only small MS parameter alterations. The UQ\textsubscript{m}/MK\textsubscript{m} ratio can be used to access the redox state for a short-term history (weeks) of oxygen availability in the presence of Gram-negative aerobic, facultative aerobic or anaerobic microorganisms.

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