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Research Paper

Biosynthesis of arsenolipids by the cyanobacterium *Synechocystis* sp. PCC 6803

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Environmental context. Arsenic biotransformation processes play a key role in the cycling of arsenic in aquatic systems. We show that a freshwater cyanobacterium can convert inorganic arsenic into arsenolipids, and the conversion efficiency depends on the arsenic concentration. The role of these novel arsenic compounds remains to be elucidated.

Abstract. Although methylated arsenic and arsenosugars have been verified in various freshwater organisms, lipid-soluble arsenic compounds have not been identified. Here, we report investigations with the model organism cyanobacterium *Synechocystis* sp. PCC 6803 wild type and Δ*arsM* (arsenic(III) *S*-adenosylmethionine methyltransferase) mutant strain, which lacks the enzymes for arsenic methylation cultured in various concentrations of arsenate (As^V). Although *Synechocystis* accumulated higher arsenic concentrations at the higher exposure levels, the bioaccumulation factor decreased with increasing As^V. The accumulated arsenic in the cells was partitioned into water-soluble and lipid-soluble fractions; lipid-soluble arsenic was found in *Synechocystis* wild type cells (3–35 % of the total depending on the level of arsenic exposure), but was not detected in *Synechocystis* Δ*arsM* mutant strain showing that ArsM was required for arsenolipid biosynthesis. The arsenolipids present in *Synechocystis* sp. PCC 6803 were analysed by high performance liquid chromatography–inductively coupled plasma–mass spectrometry, high performance liquid chromatography–electrospray mass spectrometry, and high resolution tandem mass spectrometry. The two major arsenolipids were characterised as arsenosugar phospholipids based on their assigned molecular formulas $C_{47}H_{88}O_{14}AsP$ and $C_{47}H_{90}O_{14}AsP$, and tandem mass spectrometric data demonstrated the presence of the phosphate arsenosugar and acylated glycerol groups.

Additional keyword: arsenic.

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Introduction

Arsenic is a widely distributed toxicant in the environment. Naturally, arsenic is found in soils and waters mainly in inorganic forms in a +3 and +5 oxidation state, arsenite (As^{III}) and As^V. As a chemically active element, arsenic can affect physiological and biochemical processes of living organisms. Organisms have developed specific metabolic pathways to detoxify arsenic in the environment. Many freshwater organisms can reduce As^V to As^{III}, which is then pumped out by membrane proteins, thereby decreasing the arsenic content in cells. Marine organisms tend to use biomethylation as an arsenic detoxification mechanism leading to an accumulation of organic arsenic compounds. The most abundant and common arsenic compounds are arsenobetaine, the major arsenical in marine animals, and arsenosugars, which are dominant in marine algae.[1] Arsenobetaine and arsenosugars have also been identified in freshwater[2–4] and terrestrial organisms,[5] but generally only at low concentrations.

Over the last few years, interest has turned to the investigation of arsenic-containing lipids (arsenolipids) and the possible role they might play in the transformation and detoxification of arsenic. Although the presence of arsenic in lipid fractions of fish (cod liver oil and herring oil) was first reported in 1968,[6] the structures of these lipids have mostly been elucidated only over the last 6 years. Thus, arsenic-containing fatty acids were identified in cod liver oil[7] and capelin fish meal,[8] and the

closely related arsenic-containing hydrocarbons were found in capelin oil,[9] cod liver[10] and sashimi tuna.[11] A third class of arsenolipid, namely arsenosugar phospholipid, has been identified in marine algae.[12–14] Although arsenolipids are now established as common arsenic metabolites in marine animals and algae, their presence in freshwater organisms has not been demonstrated, and knowledge concerning the biological relevance and biosynthesis mechanism of the arsenolipids is still limited.

A recent report[4] that the cyanobacterium *Synechocystis* sp. PCC 6803 is capable of producing arsenosugars has encouraged us to explore arsenic metabolism and genetic manipulation in this organism. *Synechocystis* sp. PCC 6803 is an oxygenic photo-autotrophic unicellular cyanobacterium, the entire genome of which has been sequenced.[15,16] *Synechocystis* sp. PCC 6803 is widely used as a model organism to study molecular mechanisms of biological metabolism because of the ease with which it can be genetically modified.[17] In a preliminary study, we found that ArsM was required for arsenosugar biosynthesis in *Synechocystis* sp. PCC 6803 (X. M. Xue, J. Ye, G. Li, H. Gao, C. Rensing, Q. Q. Chi, K. A. Francesconi, Y. G. Zhu, unpubl. data). Here, we investigate whether *Synechocystis* sp. PCC 6803 can produce arsenosugar arsenolipids and to identify if ArsM is involved in arsenolipid biosynthesis.

Materials and methods

Cyanobacteria culture and harvesting

Axenic cultures of *Synechocystis* wild type (WT) and *Synechocystis* Δ*arsM* were grown in 250-mL Erlenmeyer flasks containing 100 mL of BG-11 medium[18] at 30 °C with shaking at 96 rpm under a 12–12 light cycle (40 μmol photons m^{-2} s^{-1}) for 3 weeks. BG-11 medium was adjusted to pH 7.6 with 25 % HNO_3 (Merck, Darmstadt, Germany) after adding 0.1–100 μL of 100 mM Na_2HAsO_4 (BZL, Beijing, China); each treatment was cultured in triplicate. For all the *Synechocystis* Δ*arsM* cultures, at least 50 μg mL^{-1} of the antibiotic kanamycin (Solarbio, Beijing, China) was added to the culture medium. Cells were grown to stationary phase, and cultured cells were diluted with fresh sterile medium to give an optical density at 730 nm ($OD_{730\ nm}$) of 0.1. After 3 weeks, ~50 mL of the cyanobacteria was harvested by centrifuging at 3220*g* and room temperature 25 °C for 10 min, and the cells were transferred to 15-mL polypropylene tubes with screw-caps after being washed three times with BG11 medium. The cells were broken by subjecting them to ten cycles of freezing in liquid N_2 and thawing at 37 °C, and then freeze-dried. For the cells sent to Graz for characterisation of the arsenolipids, *Synechocystis* WT and *Synechocystis* Δ*arsM* were cultured in 2-L Erlenmeyer flasks containing 500 mL of BG-11 medium and 1 μM As^V at 30 °C.

Fractionation of arsenic in Synechocystis *sp. PCC 6803*

The lyophilised cyanobacteria cells were mixed with 10 mL of $CHCl_3/CH_3OH$ (Fisher Scientific, Fair Lawn, NJ, USA) (2 : 1, v/v), vortexed for 1 min to assist mixing, and placed on a rolling incubator (Kylin-Bell laboratory Instruments Co., Ltd, Haimen, Jiangsu, China) overnight. The separation of the water-soluble and lipid-soluble arsenic was carried out as described by Thomson et al.[19] Samples were then centrifuged for 10 min at 3220*g* and room temperature 25 °C to separate residue and supernatant, and the supernatants were pipetted into 50-mL polypropylene vials. The residue was extracted again and centrifuged, and the second supernatant was combined with the first. In addition, the lipid-soluble arsenic was separated into polar arsenolipids and non-polar arsenolipids by adding 4 mL of hexane and 3 mL of CH_3OH/H_2O (9 : 1, v/v) according to the previously described method.[11]

Determination of total arsenic by inductively coupled plasma–mass spectrometry (ICP-MS) – Xiamen

Total arsenic concentrations were determined using an Agilent 7500cx ICPMS (Agilent Technologies, Santa Clara, CA, USA) operated in collision cell mode. The masses measured were ^{75}As, ^{72}Ge and ^{103}Rh, with the last two masses serving as a check for signal stability. To monitor instrument drift, a standard solution of 10-μg L^{-1} arsenic was analysed every 20 samples, and the results were accepted only if the value of this 'drift standard' remained within 20 % of the mean value over the course of the analyses. The analytical method was validated through analyses of GBW10025 (spiral algae) supplied by the National Institute of Metrology, P. R. China (certified [total As] = 0.22 ± 0.03 mg As kg^{-1}), found [total As] = 0.24 ± 0.06 mg As kg^{-1} ($n = 4$).

Arsenic speciation analysis – Xiamen

High performance liquid chromatography (HPLC)/ICPMS measurements were performed using an Agilent 7500cx ICPMS for element-selective detection coupled with an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved on a Hamilton PRP-X100 anion-exchange column (4.1 × 250 mm, 10 μm) with matching guard column (Hamilton Co., Reno, NV, USA). The mobile phase containing 15 mM $NH_4H_2PO_4$ (Alfa Aesar, Ward Hill, MA, USA) at pH 5.6 was pumped through the column at 1.5 mL min^{-1}. The injection volume was 100 μL and the column temperature was 40 °C. The ICPMS was tuned to monitor *m*/*z* 75 (arsenic); at the same time, *m*/*z* 77 and 82 (selenium) were monitored to verify that ArCl interferences were not present. Arsenic species were identified by retention time matching with standard arsenicals and by spiking experiments. In addition, *m*/*z* 72 (germanium) and *m*/*z* 103 (rhodium) were used as internal standards; a standard solution of 10 μg As L^{-1} as dimethylarsinic acid (DMA^V, Accustandard, New Haven, CT, USA) was used as a drift standard introduced at a frequency of one injection every twenty samples. The various species were quantified by external calibration against DMA^V.

Determination of polar arsenic species in Synechocystis *cells – Graz*

Extraction of polar arsenic species

Approximately 10 mg of freeze-dried cells was weighed with a precision of 0.01 mg into 1.5-mL Eppendorf tubes, and an aqueous solution (1 mL) of 10-mM malonic acid (>98 %) from Fluka (Buchs, Switzerland), adjusted to pH 5.6 with aqueous ammonia (25 %, p.a., Fluka), was added. Samples were extracted in an ultrasonic bath for 15 min followed by overnight shaking. The extracts were centrifuged for 15 min at 8900*g* and 4 °C. The supernatant was used directly for HPLC/ICPMS analysis. To a separate aliquot of the extracts (200 μL), 20 μL of hydrogen peroxide (30 %, p.a.) from Carl Roth GmbH (Karlsruhe, Germany) was added in order to oxidise As^{III} to As^V, and thio-As species to oxo-As species.

Extraction of less polar arsenic species

Approximately 50 mg of freeze-dried cells were weighed (to a precision of 0.1 mg) directly into a centrifuge tube (10 mL, polypropylene), 5 mL of a mixture of CH_3OH/H_2O (9 : 1, v/v) was added, and the tubes were rotated on a rotary wheel

overnight. A portion of the mixture was syringe filtered (0.2 μm) into a HPLC vial before analysis by HPLC/ICPMS. These extraction conditions are suitable for extracting many organoarsenic species, including arsenosugars, but the highly polar As^V is poorly extracted.

Ion-exchange HPLC/ICPMS analysis of polar arsenic species

Anion-exchange HPLC was performed at 40 °C with a PRP-X100 column (4.1 × 150 mm; 5-μm particle size; Hamilton Co., Reno, NV, USA) and a mobile phase of malonic acid (5 or 10 mM at pH 5.6, adjusted with aqueous ammonia) under gradient elution conditions: 0–3 min, 5 mM; 3–9 min, 10 mM; then 9–12 min, 5 mM to re-equilibrate the column before the next injection. The flow rate was 1 mL min^{-1}, the injection volume was 20 μL. Cation-exchange HPLC was performed at 30 °C with an Ionospher 5C column (3 × 100 mm, Agilent, Waldbronn, Germany) and a mobile phase of 10-mM aqueous pyridine (Merck) adjusted to pH 2.5 with formic acid. The flow rate was 1 mL min^{-1}; the injection volume was 20 μL. Arsenic species were identified by retention time matching and spiking experiments with standard arsenic species as previously reported.[20] Quantification was performed by external calibration against DMA^V.

Determination of arsenolipids in *Synechocystis* cells

Extraction of arsenolipids

A portion (50 mg, weighed to a precision of 0.1 mg) of the freeze-dried cells was weighed into a centrifuge tube and extracted with a mixture of $CHCl_3/CH_3OH$ (1 : 2, v/v) overnight on a rotary wheel. The mixture was centrifuged; 4.5 mL of the supernatant was transferred to a clean tube containing 0.5 mL of a 1 % aqueous $NaHCO_3$ solution (Fluka) and the mixture was gently shaken. A portion of the $CHCl_3$ layer was applied to a small column of silica (conditioned with 1 % formic acid in acetone/$CHCl_3$, 1 : 1, v/v) packed into a Pasteur pipette. The silica column was washed with CH_3OH (5 mL) and then CH_3OH containing 1 % ammonia whereupon the arsenolipids were eluted. This arsenolipid fraction was evaporated to dryness, and the residue was re-dissolved in CH_3OH (200 μL) before HPLC/ICPMS–electrospray ionisation mass spectrometry (ESIMS) (Agilent Technologies, Waldbronn, Germany) analysis.

HPLC/ICPMS and HPLC/ESIMS analysis of arsenolipids

Separations were performed with an Agilent Zorbax C8 column (150 × 4.1 mm, 5 μm, Agilent SA, Basel, Switzerland) and a mobile phase run at the following gradient elution conditions: eluent A, 0.1 % formic acid in water; eluent B, 0.1 % formic acid in CH_3OH; gradient, 0–15 min 5–95 % B, 15–32 min constant 95 % B, 32 min 5 % B, 32–40 min constant 5 % B; flow, 1.0 mL min^{-1}; injection volume, 5 μL. For ICPMS detection, the HPLC was carried out with an Agilent 1100 HPLC system connected to an Agilent 7500ce ICPMS (both from Agilent Technologies, Waldbronn, Germany). To prevent carbon deposition on the interface cones, an optional gas (1 % oxygen in argon) was introduced. The nominal mass of the molecules containing arsenic were recorded by splitting the effluent flow from the HPLC column and simultaneously determining arsenic by ICPMS and protonated molecular ions by electrospray single quadrupole MS, as previously described.[13]

For electrospray high-resolution MS measurements, the mobile phase was delivered by a Dionex Ultimate 3000 system (Thermo Scientific, Bremen, Germany). Full spectra and tandem mass spectra were obtained with a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific); source, HESI2, positive ion mode; spray voltage, 3.2 kV; capillary temperature, 320 °C; sheath gas, 75 au (arbitrary units); aux gas, 20 au; temperature, 500 °C; settings for full scan *m/z* 150–2000; resolution, 70 000 (full width at half maximum height, FWHM); automatic gain control (AGC) target, 3×10^6; maximum inject time, 200 ms. Data Dependent MS2: full scan *m/z* 100–1100; resolution 70 000 (FWHM); AGC target 3×10^6; maximum inject time, 100 ms; the five most intense peaks were selected; dynamic exclusion time 5 s. Tandem mass spectrometry (MSMS) settings: isolation window 1 Da; normalised collision energy, 30 au; resolution 17 500 FWHM; AGC target, 1×10^5; maximum inject time, 50 ms. The simulated spectra were obtained with *Xcalibur* software (Thermo Scientific).

Results

Arsenic speciation in the medium

Arsenic speciation in the culture medium was investigated after the cyanobacteria were cultured for 3 weeks (Fig. 1). In *Synechocystis* Δ*arsM* medium, only inorganic As was detected for all treatments, with As^{III} being the predominant species (>50 %) in the medium initially containing As^V at concentrations of 1, 10 or 100 μM. For the medium from the *Synechocystis* WT cultures, however, organoarsenicals (methylarsonic acid (MA) + DMA + arsenosugars) were significant at the 0.1 μM As^V exposure constituting 34 % of the total arsenic (Fig. 1). At the high exposures, As^{III} was the dominant species, and organoarsenicals were either not detected or present at only trace levels.

Arsenic accumulation in cells

The arsenic concentrations in *Synechocystis* WT and *Synechocystis* Δ*arsM* exposed to various As^V concentrations are summarised in Table S1 of the Supplementary material. Both *Synechocystis* types showed a strong capability to accumulate arsenic from the medium, although *Synechocystis* Δ*arsM* cells accumulated slightly less arsenic than did *Synechocystis* WT cells. Increasing arsenic concentration in the medium led to greater arsenic accumulation in the cells – as arsenic exposure increased from 0.1 to 100 μM As^V, the total arsenic concentration in *Synechocystis* WT cells increased from 31.3 to 1293 mg kg^{-1} whereas the total arsenic accumulated by *Synechocystis* Δ*arsM* cells increased from 10.1 to 1067 mg kg^{-1}. However, relative concentration factors[21] (expressed as milligrams of As per kilogram of dry mass of cells per exposure concentration in milligrams per kilogram) decreased markedly at higher arsenic exposures. After exposure to As^V for 3 weeks, the concentration factors were 1505 for *Synechocystis* WT and 486 for *Synechocystis* Δ*arsM* in the 0.1 μM As^V treatment, whereas for the 100 μM As^V treatment, the concentration factors were only 62 and 51 for *Synechocystis* WT and *Synechocystis* Δ*arsM*.

Fractionation of arsenic in Synechocystis

The total arsenic of the cyanobacteria cells was fractionated into water-soluble, lipid-soluble, and residue arsenic by extracting and partitioning between chloroform and water. Only the *Synechocystis* WT produced significant amounts of lipid-soluble arsenic, and the relative proportions decreased with increasing arsenic exposure (Table S1). For example, at a low exposure of 0.1 μM As^V, ~35 % of the total arsenic was lipid-soluble whereas at high exposure of 100 μM As^V, only ~3 % was lipid-soluble. Arsenolipids constituted 27 % of the total arsenic in *Synechocystis* WT cells treated with 1 μM As^V; the percentage of arsenolipids reduced to 6 % when the exposure

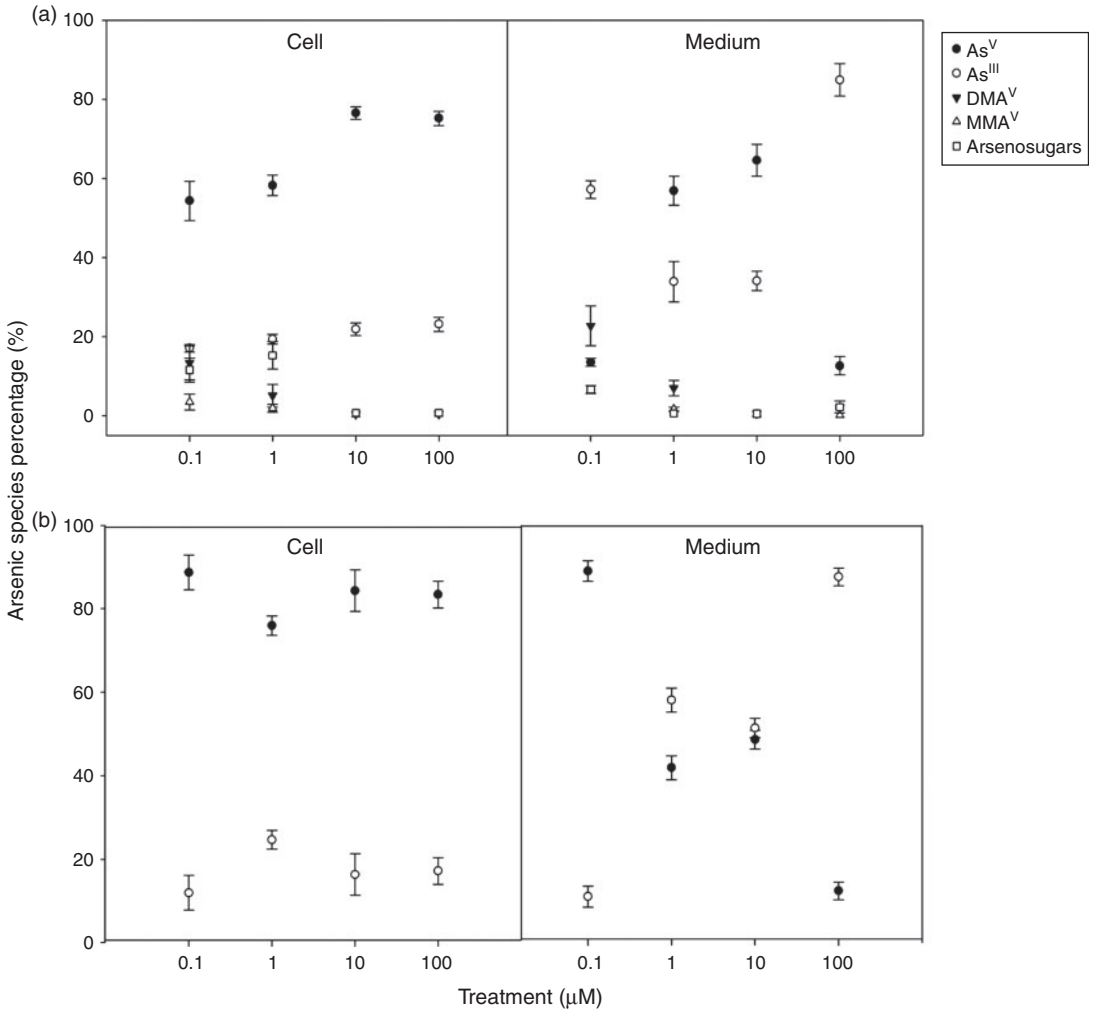

Fig. 1. Arsenic species percentages in *Synechocystis* sp. PCC 6803 cells (WT (a) and Δ*arsM* (b)) (approx. percentage of total water-soluble arsenic) grown in medium containing different arsenic concentrations. Shown is the mean of three independent culture experiments (DMA, dimethylarsinic acid; MMA, monomethylarsonic acid).

level was 10 μM As^V. These data are consistent with the arsenolipid biosynthesis pathway being overloaded at higher As^V exposures. The lipid-soluble arsenic compounds were further fractionated into non-polar and polar arsenolipids by partitioning between hexane and methanol. No arsenic was found in the hexane layer thereby demonstrating that *Synechocystis* sp. PCC 6803 was not able to produce non-polar arsenolipids.

As^V dominates in water-soluble arsenic of cells

Arsenic biotransformation in *Synechocystis* sp. PCC 6803 cells exposed to different As^V concentrations was investigated (Fig. 1). For *Synechocystis* Δ*arsM* cells cultured under all treatments, only As^{III} and As^V were found in the cells. In contrast, the cells from *Synechocystis* WT showed significant amounts of organoarsenicals, particularly at the low As^V exposures. The concentrations of arsenic species in the cells over the range of exposures were 54–77 % As^V, 17–23 % As^{III}, 0.4–13 % DMA, 0.4–3.5 % MA, and 0.7–15 % arsenosugars. Organoarsenic compounds accounted for 28.5 % of the total As species for 0.1 μM As^V treatment, whereas, the proportion of organoarsenicals was reduced to 1.5 % for the higher As^V treatments (≥10 μM).

In subsequent replicate experiments, *Synechocystis* WT and *Synechocystis* Δ*arsM*, were individually cultured in quadruplicate. The simple methylated arsenicals MA and DMA, and three arsenosugars (glycerol, phosphate and sulfonate; see Supplementary material, Fig. S1 for structures) were produced only by *Synechocystis* WT. *Synechocystis* Δ*arsM* did not produce any detectable organoarsenic species (<10 μg As kg^{-1}). The amounts of organoarsenicals produced by *Synechocystis* WT expressed as micrograms of As per kilogram (mean ± s.d., $n = 4$) of dry cells were: MA (155 ± 28); DMA (215 ± 91); glycerol arsenosugar (54 ± 17); phosphate arsenosugar (675 ± 246) and sulfonate arsenosugar (389 ± 144). The sulfate arsenosugar was present in *Synechocystis* WT, but only at trace, non-quantifiable levels (<20 μg As kg^{-1}). The amounts of the organoarsenic compounds in *Synechocystis*, expressed as a percentage of the As^V content, were: MA (0.8 %), DMA (1.2 %), glycerol arsenosugar (0.25 %), phosphate arsenosugar (3.5 %), and sulfonate arsenosugar (2.0 %). These values are highly dependent on the extraction method, and refer to the relative amounts of arsenicals extracted into water. For example, when CH_3OH/H_2O (9 : 1, v/v) was used as extractant, the organoarsenic species in the extract predominated over the As^V owing to the poor methanol solubility of As^V.

Synechocystis *sp. PCC 6803 produces arsenosugar phospholipids*

For identification of arsenic compounds in the lipid fraction of *Synechocystis* WT, a clean-up procedure with silica was

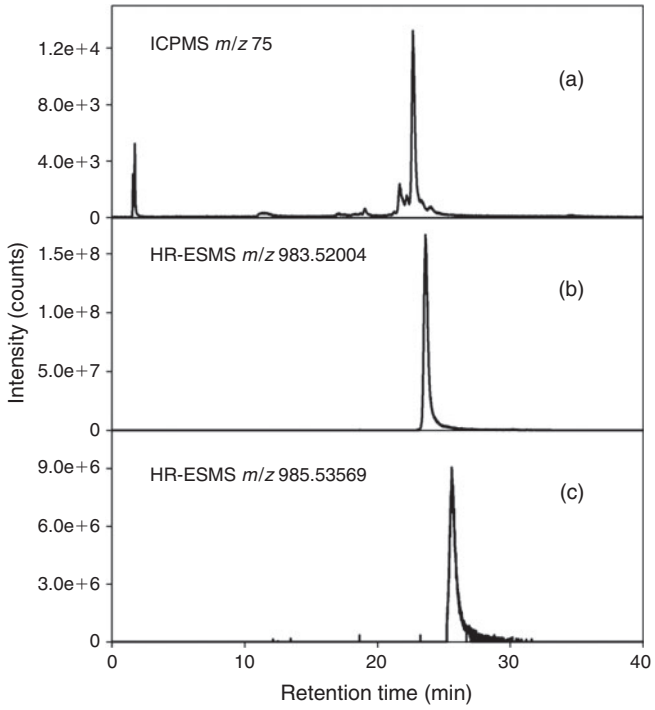

Fig. 2. High performance liquid chromatography/mass spectrometry (HPLC/MS) chromatograms of the lipid extract (post-silica column) of *Synechocystis* WT. (a) Inductively coupled plasma–mass spectrometry (ICPMS) detection at *m/z* 75 (arsenic-selective); ions selected with a 4-ppm tolerance centred on (b) *m/z* 983.52004 (retention time (RT) = 23.32 min; $C_{47}H_{88}O_{14}AsP\ H^+$) and (c) 985.53569 (RT = 25.17 min; $C_{47}H_{90}O_{14}AsP\ H^+$). Separations were performed on an Agilent Zorbax C8 column (150 × 4.1 mm, 5 μm) and a mobile phase run at the following gradient elution conditions: eluent A, 0.1 % formic acid in water; eluent B, 0.1 % formic acid in CH_3OH. Gradient: 0–15 min 5–95 % B, 15–32 min constant 95 % B, 32 min 5 % B, 32–40 min constant 5 % B; flow was 1.0 mL min^{-1}; injection volume was 5 μL. The retention times differ slightly between arsenic-selective detection and compound detection owing to small differences in the 'plumbing' between the ICPMS and the high resolution electrospray MS (HR-EMS).

invoked. HPLC/ICPMS analysis of the post-silica lipid fraction from *Synechocystis* WT showed the presence of a major arsenolipid and several minor ones (Fig. 2a); this chromatographic behaviour was similar to that found previously for arsenosugar phospholipids.[13] Investigation by HPLC/ESIMS revealed that the major arsenic compound seen by HPLC/ICPMS had $[M + H]^+$ 983, and a minor compound had $[M + H]^+$ 985 (Fig. 2a, b). Accurate mass measurements suggested the molecular formulas $C_{47}H_{88}O_{14}AsP$ (Anal. Calc. for $[M + H]^+$ 983.5200; found: 983.5182, $\Delta m = -1.8$ ppm) and $C_{47}H_{90}O_{14}AsP$ (Anal. Calc. for $[M + H]^+$ 985.5357; found: 985.5324, $\Delta m = -3.3$ ppm). Moreover, the isotope patterns from these ions matched exactly the simulated patterns for these molecular formulas (see Supplementary material, Figs S2, S3). MSMS performed on these two ions identified the fragments corresponding to the deacylated phosphate arsenosugar and the acylated glycerol group (Fig. 3, Table 1). Collectively, these data strongly support the assignment of the two structures as shown in Fig. 4.

Discussion

Arsenic in the model organism cyanobacterium *Synechocystis* sp. PCC 6803 was distributed between water- and lipid-soluble components. The majority of the extractable arsenic in cells was As^V for all treatments with smaller amounts of As^{III}, DMA^V, MA^V, and arsenosugars being identified in wild type cells. Compared to other studies[4,22] with cultures of *Synechocystis* sp. PCC 6803, our results showed less DMA^V or arsenosugars when cells were exposed to 100 μM As^V. These differences may be due to sample extraction methods. Yin et al.[22] prepared samples using 1 % HNO_3 and microwave treatments, in which the original arsenic species were chemically degraded to DMA^V.[23] Miyashita et al.[4] extracted samples by sonication with water. Arsenosugars may be degraded to DMA^V during sonication.[2] Miyashita et al.[4] cultured *Synechocystis* sp. PCC 6803 for 24 h, and found that the level of phosphate arsenosugar was low and fluctuated throughout the experiment. This variability might be because phosphate arsenosugar can be derived, at least in part, from the degradation of arsenosugar phospholipids. In that regard, arsenosugar phospholipids might be the actual target arsenic compounds synthesised by algae.[13]

Reduction and efflux of arsenic are of considerable significance in arsenic detoxification by microorganisms.[24] The results from our study illustrated the capacity of the cyanobacterium *Synechocystis* sp. PCC 6803 to perform arsenic uptake, reduction and excretion. *Synechocystis* sp. PCC 6803 has a robust ability to accumulate arsenic, but a low ability to synthesise organoarsenicals (Table 1). The ability of *Synechocystis* to accumulate arsenic decreased with increasing arsenic concentration in the medium. For example, the bio-concentration factor decreased from 1505 to 62 when arsenic exposure increased from 0.1 to 100 μM (Table 1) for *Synechocystis* WT. Similar results have also been found for the green alga *Chlorella vulgaris*,[25,26] the marine cyanobacterium *Phormidium* sp.[27] and the freshwater fish, *Tilapia mossambica*.[28] In contrast, the uptake of arsenic by the two marine algae *Fucus spiralis* and *Ascophyllum nodosum* remained constant at external concentrations of As^V exceeding 1000 μg kg^{-1}.[29]

Synechocystis WT was able to change the species of arsenic in the medium through absorbing As^V and subsequently excreting arsenic in a reduced form (at higher As^V exposure) or in organic forms (at low As^V exposure). For *Synechocystis* Δ*arsM*, which lacks the ability to methylate arsenic, the majority of the arsenic in the medium was As^{III}. Microbial uptake of As^V can be mediated by the phosphate transport system owing to the chemical similarity of arsenate to phosphate.[30,31] The cyanobacterium took up As^V after exposure to various concentrations of As^V, and the arsenic was subsequently reduced to As^{III} by ArsC.[32,33] As^{III} is more toxic than As^V for it can impair physiological function by binding thiol groups in proteins.[24] To counteract the deleterious effects of As^{III}, *Synechocystis* sp. PCC 6803 evolved with three resistance strategies, including As^{III} re-oxidation,[22] arsenic methylation into less toxic species and active extrusion of As^{III} from the cells by ArsB, an As^{III} carrier. Previous studies[34–36] have shown that organisms could alleviate As^{III} toxicity by extruding As^{III} into their external environment. In this study, more As^{III} was observed in the medium than in cells when the cyanobacterium was exposed to As^V, indicating that As^{III} efflux might be the most important step among the three arsenic detoxification strategies.

The organoarsenicals occur in a wide range of marine and freshwater environments where they play a key role in arsenic cycling. Marine algae could transform As^V into organoarsenic compounds (MA, DMA, arsenosugars and arsenolipids) in seawater low in phosphate. The levels of arsenic in seawater

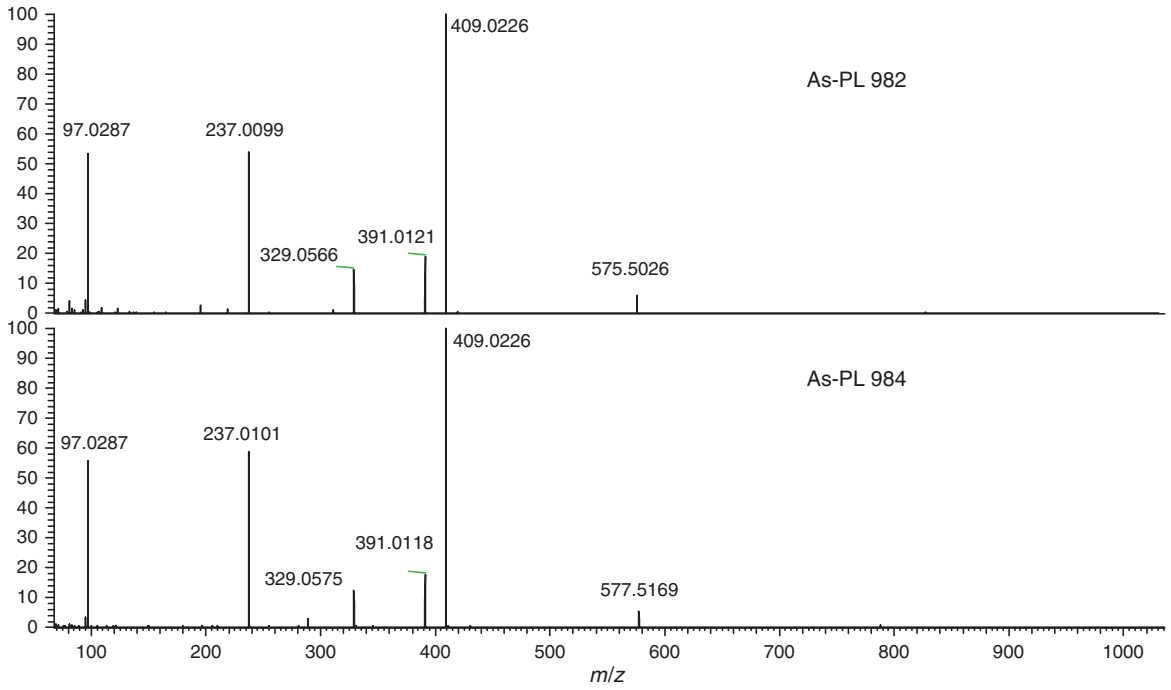

Fig. 3. Tandem mass spectrometry spectra of two arsenosugar phospholipids produced by *Synechocystis* WT.

Table 1. Tandem mass spectrometry data for two arsenosugar phospholipids (As-PL) produced by *Synechocystis* WT

Fragment	Fragment *m/z* calculated	As-PL 982 fragment *m/z* found (Δ*m*, ppm)	As-PL 984 fragment *m/z* found (Δ*m*, ppm)
$C_5H_5O_2^+$	97.0284	97.0287 (3.1)	97.0287 (3.1)
$C_7H_{14}O_4As^+$	237.0102	237.0099 (−1.3)	237.0101 (−0.4)
$C_{10}H_{22}O_7As^+$	329.0576	329.0566 (−3.0)	329.0575 (−0.3)
$C_{10}H_{21}O_9AsP^+$	391.0133	391.0121 (−3.1)	391.0118 (−3.8)
$C_{10}H_{23}O_{10}AsP^+$	409.0239	409.0226 (−3.2)	409.0226 (−3.2)
$C_{37}H_{67}O_4^+$	575.5034	575.5026 (−1.4)	–
$C_{37}H_{69}O_4^+$	577.5190	–	577.5169 (−3.6)

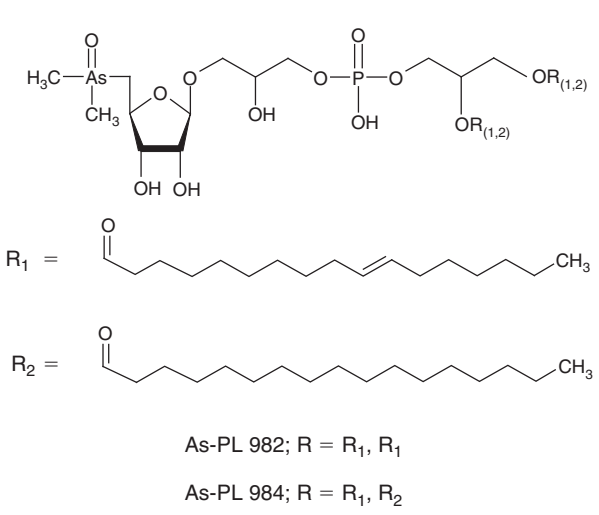

Fig. 4. Two arsenosugar phospholipids identified in the cyanobacterium *Synechocystis* sp. PCC 6803. The 5C sugar is ribose. The position of the double bond is unknown (mass spectrometric fragmentation did not elucidate its position); it is drawn as 10-heptadecenoic acid.

normally range between 0.5 and 2 μg kg^{-1},[37] values comparable to phosphate levels in low phosphate waters. Low concentrations of phosphate were found to be a primary factor regulating the transformation of As^V by algae.[38] Possibly, marine algae may utilise arsenic in place of P to form membrane lipids.[13] Freshwater cyanobacteria synthesise lower quantities of organoarsenicals than do marine algae, perhaps because of the higher levels of phosphate in freshwater. Our study showed that the biosynthesis of organoarsenicals is not the primary detoxification pathway in *Synechocystis* sp. PCC 6803 at high As^V exposure. In the process of evolution, freshwater cyanobacteria retained several arsenic transformation pathways of marine algae, but may ultimately utilise arsenic oxidation, reduction and extrusion as the major detoxification pathways to save energy.

Although *Synechocystis* matched marine algae by being able to biosynthesise arsenosugar phospholipids, it differed in that the major arsenolipid was not the palmitic acid derivative found in marine algae.[12–14] Rather, the major compound contained saturated and unsaturated 17-carbon groups on the glycerol moiety. More data are required to determine if these differences have some chemotaxonomic significance.

In summary, our study showed that when exposed to As^V the model organism *Synechocystis* sp. PCC 6803 produces arseno-sugar phospholipids, and that ArsM is a required enzyme in the biosynthesis of these lipids. We hope that our investigation with *Synechocystis* sp. PCC 6803 will stimulate more studies on the biosynthesis of arsenolipids, leading to an understanding of the possible function of these unusual lipids in biological systems.

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