Clipboard
DOI 10.1007/s12038-011-9076-3

Controversy over the report on a bacterium that feeds on arsenic

Arsenic (As), the metalloid, has been traditionally infamous for its toxicity. Because of its use by rulers to kill their rivals, it was once known as the poison of the kings or the king of poisons. It was also widely used as an agricultural insecticide. It kills humans quickly if consumed in large quantity. Also, regular small doses of arsenic cause various illnesses, such as patchy skin, digestive system disorders, peripheral neuritis, hepatic lesions and fatty degeneration of the heart, and other life-threatening complications. Exposure to arsenic could be due to both natural and anthropogenic factors.

In nature, arsenic occurs in four oxidation states: As(V), As(III), As(0) and As(−III). Occurrence of the highest oxidation states is more common and that of the two lowest oxidation states is rare. In aqueous aerobic environments, arsenate (AsO_4^{-3}), the pentavalent form, predominates. It tends to be strongly adsorbed onto common minerals (e.g. alumina). Arsenite (AsO_3^{-3}), the trivalent form, is more prevalent in anoxic environments and is substantially more toxic than arsenate. By virtue of its ability to bind sulphydryl groups, arsenic binds and inactivates enzymes. It inhibits the enzyme pyruvate dehydrogenase, essential for oxidation of pyruvate to acetyl-Co-A. In the process, it triggers cellular apoptosis. It also binds dithiols such as glutaredoxin and stimulates the production of hydrogen peroxide, thus leading to increase in oxidative stress. Inorganic arsenic trioxide, found in groundwater, affects voltage-gated potassium channels, thus creating neurological disturbances. This metabolic interference ultimately leads to death due to multiorgan failure. In the periodic table, arsenic is positioned just below phosphorus. Because of their similarity in electronegativity and radii, arsenate ion can replace phosphate in biological reactions. Thus, it can enter the early stages of metabolism, but fails to continue metabolism because of the rapid hydrolysis of the arsenate esters, compared with that of the phosphate esters. The hydrolysis of diesters is faster than that of triesters (Westheimer 1987). Arsenate esters are labile even if very low concentration of water is present. Presence of 0.5% water allows a half-life of less than 0.1 s at pH 9.0. Compared with phosphodiester-containing DNA, which has a half-life of 3×10^7 years, arsenodiester-containing DNA spontaneously hydrolyses with an estimated half-life of 0.06 s at 25°C (Fekry *et al.* 2011).

Analysis of arsenic-rich water and soil, sampled from different places, have so far revealed the presence of several types of bacteria belonging to the genera *Acidithiobacillus*, *Bacillus*, *Deinococcus*, *Desulfitobacterium* and *Pseudomonas* (Shivaji *et al.* 2005 and the references therein). These bacteria can tolerate arsenate in the presence of phosphate. The genes responsible for arsenic tolerance in these organisms are organized in an operon, called *ars* operon. Some bacteria can use arsenic as an electron donor; others can methylate inorganic arsenic or demethylate organic arsenic compounds. *Bacillus macyae* is an example of arsenate-respiring bacteria (Páez-Espino *et al.* 2009). However, until a couple of months ago, no bacterium was found to tolerate arsenic in absence of phosphorus. In fact, no form of life that could utilize arsenic was known to occur.

In 2009, Felisa Lauren Wolfe-Simon, a NASA research fellow at the US Geological Survey, and her two colleagues postulated for the first time that arsenic might substitute phosphorus in ancient living systems (Wolfe-Simon *et al.* 2009). The postulation failed to evoke any response from the scientific community, the main reason being the instability of arsenate esters. Wolfe-Simon, however, stuck to her idea, and in order to pursue it, she collected mud samples from Mono Lake, a salty water body in California with high arsenic content. She started enriching it with bacterial growth medium containing carbon source, vitamins and trace metals with increasing concentrations of arsenate but no phosphorus. Several decimal-dilution transfers were performed to reduce any carryover from the phosphorus indigenous to the sample. Ultimately she reached a stage when the amount of phosphorus present in the

Keywords. Arsenic; arsenate esters; arsenate-utilizing; toxicity; Wolfe-Simon

medium (3.1 μM) was not sufficient to allow growth of any bacterium. Hence, she was highly surprised when she saw some organisms in the culture medium examined under a microscope. The isolate (named GFAJ-1) belonged to the Halomonadaceae family of γ-Proteobacteria, which could be maintained aerobically with 40 mM arsenate, 10 mM glucose and no added phosphorus.

When grown in the presence of phosphorus, GFAJ-1 utilized 30-fold more phosphate compared with the same organism grown in the +As/−P condition. Inductively coupled plasma mass spectrometry (ICP-MS) revealed very little intracellular phosphorus in the +As/-P condition. Hence, it appeared that arsenate did not replace the requirement for phosphate when phosphate was present, but it could do so in a phosphate-deprived environment.

Distribution of arsenate in the cell, determined using radiolabelled arsenate ($^{73}AsO_4^{3-}$) in the medium, was consistent with the distribution of phosphate determined earlier. High-resolution secondary ion mass spectrometry (Nano-SIMS) of genomic DNA extracted from the organism showed higher concentration of arsenic in +As/−P DNA and higher phosphorus in −As/+P DNA.

Evidence obtained from micro–X-ray absorption near-edge spectroscopy (μXANES) and micro–extended X-ray absorption fine-structure spectroscopy (μEXAFS) indicated that intracellular arsenic was in the +5 redox state. The bond lengths of arsenic with oxygen and carbon atoms did not match with models of small arsenical molecules, but matched with the crystal structure of DNA for the analogous structural position of phosphorus with respect to oxygen and carbon. Cellular ion ratios of $^{73}As^-$:$^{12}C^-$ and $^{31}P^-$:$^{12}C^-$, determined by Nano-SIMS, confirmed the distribution of arsenic with carbon inside the cell. Besides replacing phosphorus in DNA, arsenic was also reported to be assimilated into proteins and other metabolites.

The intracellular volume of the bacterium was found to increase 1.5-fold when it was grown on arsenic as compared with that observed in the phosphorus-containing medium. Large internal compartments were also observed under a transmission electron microscope. It was postulated that these vacuole-like regions were rich in poly-β-hydroxybutyrate, which stabilized the distribution of arsenate. The postulation was bolstered by the fact that in non-aqueous environments, hydrolysis of arsenate compounds is retarded.

Despite being silent about the mechanism of incorporation of arsenic in the biomolecules, their's is the first report of an organism that not only tolerates arsenic but also incorporates it into its cell. Unlike some other organisms that use arsenic compounds as terminal electron donor, GFAJ-1 could sustain and grow in the presence of arsenic and in the absence of phosphorus (Wolfe-Simon *et al.* 2010).

This paper, which redefines the chemistry of life, has created a tumult. Appreciation as well disbelief has poured in through Twitter and journals. Some scientists such as Rosie Redfield of the University of British Columbia have written to *Science*, pointing out discrepancies in the work. Among several snags underscored by the scientific community, the purity of the genomic DNA has been called into question by many investigators. Wolfe-Simon and her team-mate Ronald Oremland have clarified that the isolated DNA passed thrice through phenol-chloroform and so it was expected to be pure. Dr Alex Bradley, a geochemist and microbiologist, has opined that the DNA could not contain arsenic as it passed through the aqueous phase during purification. He is also not convinced with Wolfe-Simon's hypothesis on stabilization of arsenic-containing DNA as he believes that such a system could not function in the absence of proteins, which were removed during purification. In reply, Wolfe-Simon published her data on the kinetics of hydrolysis of arsenic compounds and demonstrated that arsenic compounds with longer carbon chains get hydrolysed slower than those with shorter ones. Bradley has also mentioned that bacteria survive in the Sargasso Sea, where the amount of phosphate present (10 nM) is far less than the contaminating phosphate present in the medium of GFAJ-1. It has been pointed out by Tawfik and Viola (2011) that arsenate is a good substrate for L-aspartate-β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in terms of substrate affinity and catalytic efficiency, but the products are unstable. The kinetic instability in arsenic-containing DNA, as pointed out earlier, makes the concept of arsenic-dependent life unacceptable to many scientists as DNA is a biomolecule that maintains the vital genetic information through generations (Fekry *et al.* 2011).

Besides Mono Lake, arsenic is known to occur in high concentration in some other environmental niches including terrestrial and deep-sea hydrothermal systems. Organoarsenicals (e.g, trimethyl- and dimethylarsinates), arsenosugars and arsenolipids have also been found in marine organisms. Therefore, evolutionarily speaking, one can envisage a scenario with the existence of primordial arsenate-based organisms in arsenate-rich environments under dysoxic conditions and at a neutral to slightly alkaline pH, in which arsenate and arsenite are thermodynamically stable. Primordial organisms with phosphate-based

biology perhaps took over the ecosystems subsequently under aerobic conditions, restricting the arsenate-utilizing organisms to only few environments, which have remained unexplored or have not been carefully examined so far.

Regarding the future scope of this research, Jennifer Pett-Ridge, one of the co-authors of the disputed paper, said 'The team hasn't yet established how the organism uses arsenic as a building block when it's a poison to most other life forms. It could be an ancestral trait or a unique kind of metabolism'. They wish to conduct protein biochemistry in order to find out if there are specific enzymes that help transport arsenic into the cells. Chemical synthesis of arsenic-containing DNA in the laboratory and autoradiography of radioactive arsenic-labelled DNA are likely to provide further clues. Notwithstanding the scepticism and criticism, the report has earned acclamation of many scientists, who believe that a good piece of research has been done. Wolfe-Simon expects collaborations from other laboratories to discover many hitherto unknown facts about the newly discovered organism.

References

Fekry MI, Tipton PA and Gates KS 2011 Kinetic consequences of replacing the internucleotide phosphorus atoms in DNA with arsenic. *ACS Chem. Biol.* **6** 127–130

Páez-Espino D, Tamames J, de Lorenzo V and Cánovas D 2009 Microbial responses to environmental arsenic. *Biometals* **22** 117–130

Shivaji S, Suresh K, Chaturvedi P, Dube S and Sengupta S 2005 *Bacillus arsenicus* sp. nov., an arsenic-resistant bacterium isolated from a siderite concretion in West Bengal, India. *Int. J. Syst. Evol. Microbiol*. **55** 1123–1127

Tawfik DS and Viola RE 2011 Arsenate replacing phosphate: alternative life chemistries and ion promiscuity. *Biochemistry* **50** 1128–1134

Westheimer FH 1987 Why nature chose phosphates. *Science* **235** 1173–1178

Wolfe-Simon F, Blum JS, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, *et al.* 2010 A bacterium that can grow by using arsenic instead of phosphorus. *Science* **332** 1163–1166

Wolfe-Simon F, Davies PC and Anbar AD 2009 Did nature also choose arsenic? *Int. J. Astrobiol.* **8** 69–74

Dipanwita Sengupta and Madhab K Chattopadhyay*
Centre for Cellular and Molecular Biology (CSIR), Hyderabad 500 007, India
**Corresponding author (Email, mkc@ccmb.res.in)*

*e*Publication: 16 August 2011