ACS chemical biology

PERSPECTIVE

pubs.acs.org/acschemicalbiology

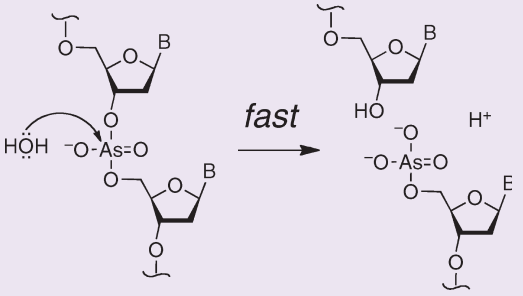
Kinetic Consequences of Replacing the Internucleotide Phosphorus Atoms in DNA with Arsenic

Mostafa I. Fekry,[†,§] Peter A. Tipton,[‡] and Kent S. Gates[†,‡,*]

[†]Department of Chemistry, 125 Chemistry Building, and [‡]Department of Biochemistry, 117 Schweitzer Hall, University of Missouri, Columbia, Missouri 65211, United States

[§]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini, Cairo, Egypt 11562

ABSTRACT: It was claimed in a recent publication that a strain of *Halomonadacea* bacteria (GFAJ-1) isolated from the arsenic-rich waters of Mono Lake, California is able to substitute arsenic for phosphorus in its macromolecules and small molecule metabolites. In this short Perspective, we consider chemical and biochemical issues surrounding the central claim that *Halomonadacea* GFAJ-1 is able to survive while incorporating kinetically labile arsenodiester linkages into the backbone of its DNA. Chemical precedents suggest that arsenodiester linkages in the putative arsenic-containing DNA of GFAJ-1 would undergo very rapid hydrolytic cleavage in water at 25 °C with an estimated half-life of 0.06 s. In contrast, the phosphodiester linkages of native DNA undergo spontaneous hydrolysis with a half-life of approximately 30,000,000 y at 25 °C. Overcoming such dramatic kinetic instability in its genetic material would present serious challenges to *Halomonadacea* GFAJ-1.



In 1987, preeminent bioorganic and physical organic chemist Frank H. Westheimer published a review article entitled "Why Nature Chose Phosphates".[1] This review is a classic in the literature of bioorganic chemistry and chemical biology because it defines the chemical principles underlying a number of central biological processes including the storage and read-out of genetic information, signal transduction, and energy transfer. More than 23 years after its publication, Westheimer's review has never been more relevant. A recently published article has caused us to reconsider the role of phosphates in biology by presenting a claim that a strain of *Halomonadacea* bacteria (GFAJ-1) isolated from the arsenic-rich waters of Mono Lake, California is able to substitute arsenic for phosphorus in its macromolecules and small molecule metabolites such as adenosine triphosphate (ATP).[2]

In this short Perspective, we consider chemical and biochemical issues surrounding the central claim in the Wolfe-Simon paper that *Halomonadacea* GFAJ-1 is able to survive using arsenic(V) rather than phosphorus(V) in its DNA, with a special focus on the kinetic instability of the proposed arsenic ester linkages in the genetic material of the microorganism.

In some regards, it is not unreasonable to speculate that arsenic(V) could replace phosphorus in the 2′-deoxyribose backbone of DNA. Indeed, some important structural and chemical similarities between phosphate and arsenate were cataloged in a 2009 article by Wolfe-Simon *et al.* entitled "Did Nature Also Choose Arsenic?" and in the recent *Science* publication.[2,3] The sizes and electronegativities of phosphate and arsenic are similar. Pauling electronegativities for P and As in the trivalent states are 2.19 and 2.18, respectively, while the atomic radius of P(V) is 0.31 Å and that of As(V) is 0.48 Å.[4,5] In addition, the pK_a values of arsenate (pK_a 2.2, 6.97, 11.53) and phosphate (pK_a 2.1, 7.2, 12.7) are quite similar.[6] Furthermore, P−O and As−O bond strengths are comparable at 143 and 115 kcal/mol, respectively.[7] From a purely structural perspective, one can envision that DNA composed of arsenodiester linkages might form a polyanionic Watson−Crick base-paired duplex that resembles normal phosphodiester-containing DNA. Supporting this view, there are many examples demonstrating that DNA analogues containing non-natural backbones can form based-paired structures resembling native duplex DNA.[8,9]

The similarities between arsenate and phosphate allow arsenate to act as a surrogate for phosphate in some circumstances, although this generally causes dysfunction and toxicity.[10,11] Bacteria have evolved elaborate systems for metabolizing arsenic in various forms and oxidation states, and arsenate enters the cells through phosphate transporters.[12] Phosphorolytic reactions such as those catalyzed by glycogen phosphorylase and sucrose phosphorylase can be supported by arsenate *in vitro*, although spontaneous hydrolysis of the arsenate monoester product dissipates the free energy that is preserved in the normal reaction. Indeed, arsenate is a substrate for ATP synthase, but the product ADP-O-AsO(OH)O^{2-} hydrolyzes spontaneously, leading to the uncoupling of oxidative phosphorylation.[13] The rapid hydrolysis

Received: January 1, 2011
Accepted: January 18, 2011
Published: January 18, 2011

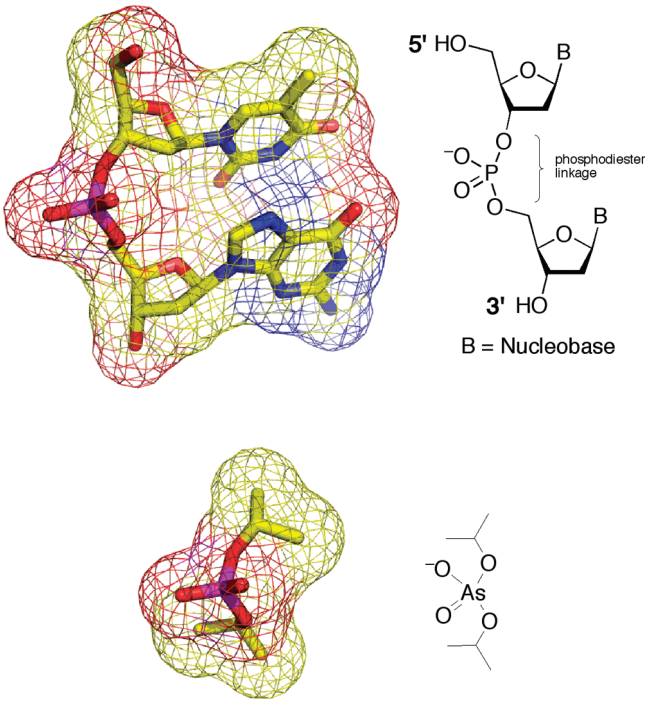
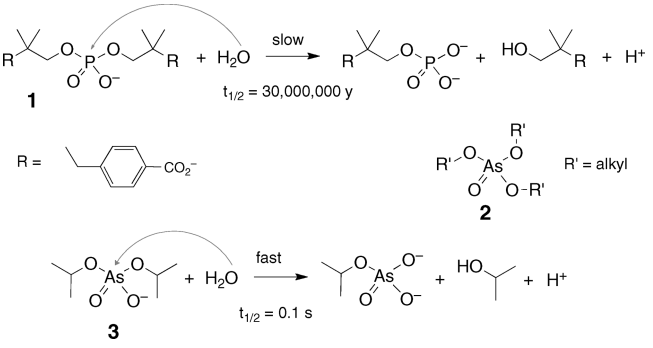


Figure 1. Steric accessibility of the phosphorus (or arsenic) center in duplex DNA is very similar to that of a diisopropyl ester model compound. The dinucleotide shown is from a segment of DNA duplex (PDB code 3BSE). The structural images were prepared using Pymol and depict both stick and surface mesh views of the molecules.

of arsenate monesters has been exploited by Schramm in his studies of purine nucleoside phosphorylases. When aresenate is subsituted for the natural substrate phosphate, the rapid decomposition of enzymatically formed ribose 1-arsenate prevents reversal of the reaction and simplifies the deconvolution of the kinetic complexity that otherwise masks the intrinsic isotope effects in the reaction.[14] The similarity between the arsenolysis reaction and the natural phosphorolysis reaction is borne out by the effectiveness of transition state analogues that were designed based on isotope effects measured in the arsenolysis reaction.[15]

However, despite the thermodynamic and structural similarities between arsenate and phosphate, the kinetic stability of arsenate diesters in water is vastly different from that of the phosphate diesters found in normal DNA. Life as we know it relies on the kinetic stability of many biomolecules, especially those involved in the storage of genetic information. We will briefly review the stability of the native phosphodiester linkages in DNA (Figure 1) before considering the properties of the proposed arsenodiesters. Breakage of the 2′-deoxyribose phosphate backbone in DNA resulting from attack of water on the phosphodiester groups (*i.e.*, hydrolysis) is thermodynamically favored ($\Delta G^{\circ\prime}$ = −5.3 kcal/mol),[16] but *extremely* slow.[17−21] Phosphodiester hydrolysis in DNA is such a sluggish reaction that the rate has been difficult to measure directly because other bonds in the biopolymer are much more labile.[21,22] In fact, one early value reported for the rate of DNA cleavage by phosphodiester hydrolysis probably represents an inadvertent measure of strand cleavage stemming from hydrolysis of the glycosidic bonds that link the nucleobases to the sugar−phosphate backbone.[23] Therefore, it has been necessary to use cleverly designed non-nucleic acid model compounds such as **1** (Scheme 1) to estimate the hydrolytic lability of DNA phosphodiester linkages.[19]

Scheme 1. Model Compounds Used To Estimate the Stability of Phosphodiester and Arsenodiester Linkages in DNA[17,24]



Even then, it was necessary to measure the rate of the hydrolysis reaction at high temperatures and extrapolate to the physiological range. The best current estimate for the half-life for hydrolytic cleavage of the DNA backbone rests at about 30,000,000 years.[19] On the basis of this rate, only about two phosphodiester linkages within the 3 billion base pairs of DNA in a human cell are expected to undergo spontaneous, uncatalyzed hydrolysis per week (in a smaller bacterial genome the number will be even lower). The immense stability of the phosphodiester linkages in DNA is a functionally important feature of the 2′-deoxyribose backbone that holds together the genetic code in all cellular organisms. Stability of the genetic blueprint is paramount: if an organism's blueprint dissolves, all is lost.

Arsenate-containing DNA has not yet been prepared and chemically characterized. Therefore, we must again turn to studies of model compounds to predict the stability of the arsenodiesters proposed by Wolfe-Simon *et al.*[2,3] To the best of our knowledge, there is only one published report that clearly addresses the stability of arsenate esters in water,[24] but the results of this study are clear: arsenate esters are cleaved very rapidly in water at RT. Edwards and co-workers used stop-flow kinetic analysis to examine the stability of several trialkyl arsenates (**2**, *R* = methyl, ethyl, *n*-pentyl, and isopropyl, Scheme 1). Work preceding Edwards' experiments had already demonstrated that this reaction involves cleavage of the As−O bonds resulting from attack of water on As (not by cleavage of the C−O bonds *via* attack at carbon).[25] The reaction rate for hydrolysis of the more sterically hindered isopropyl analogue **3** may be most relevant to the proposed arsenodiester DNA (Scheme 1). Steric accessibility of the As or P center in the DNA backbone is very similar to that in the diisopropyl ester model **3** (Figure 1). To the extent that there are differences, the increased steric bulk of the diisopropyl ester is likely to protect against hydrolysis rather than favor it. Edwards' group observed clean first-order kinetics for hydrolysis of the trialkyl arsenates.[24] This suggested that water, not hydroxide, was the attacking species in the hydrolysis reaction. In addition, there was no evidence for buildup of the arsenate diester or monoester in these experiments, suggesting that the hydrolysis of the arsenate diester is *faster* than that measured for the hydrolysis of the starting triester. The pseudo-first-order rate constant for the reaction of water (13.1 M) with triisopropyl arsenate (0.063 M) in acetonitrile was found to be 2.9 s^{-1} at 25 °C (Table 2, ref 24). This translates to an apparent second-order rate constant of 0.2 M^{-1} s^{-1} for the reaction of the arsenate ester with water. Assuming a cellular concentration 55 M water, a

pseudo-first-order rate constant of $\geq 12\ s^{-1}$ can be estimated for the intracellular hydrolysis of the arsenate diester (Scheme 1). Thus, the approximate half-life for the hydrolytic cleavage of the model arsenodiester in water at 25 °C is 0.06 s. It has been pointed out[26−29] that this level of instability is inconsistent with the reported[2] isolation of large DNA fragments constructed of arsenodiester linkages from aqueous solutions. It is noteworthy that, even if the effective concentration of water in the cellular environment were half of the 55 M used for the calculations above, the hydrolysis of the putative arsenodiesters still would be very fast − with half of the arsenodiester linkages in the microbial genome hydrolyzed in less than half a second. Finally, the hydrolytically labile nature of the As−O bonds in arsenate is reflected in the relatively fast rate of at which the oxygen of water exchanges into arsenate relative to that for phosphate.[30,31]

The stability of the proposed arsenic-containing genetic material also could be compromised by reduction and oxidation (redox) reactions that are available to arsenate. As(V) arsenate readily undergoes enzymatic conversion to As(III) arsenite under physiological conditions.[6,32−35] The chemical properties of arsenite are quite different from those of arsenate. For example, pK_a values of arsenite ($AsOH_3$, pK_a 9.1, 12.13, 13.4) suggest that an arsenite diester analogue of the DNA backbone might not be negatively charged.[6] While the exact redox properties of the proposed DNA arsenodiesters are not known, the redox activity of arsenate would likely introduce additional instability to the genetic material of an arsenophilic microorganism. In contrast, the phosphorus(V) found in the backbone of native DNA is redox-inactive under physiological conditions.

The anticipated instability of the genetic material in an arsenate-utilizing organism would present a serious challenge to cellular operations. For such an organism to survive and reproduce it would seem necessary to somehow suppress hydrolysis of the arsenodiesters. In principle, this could be accomplished by dehydrating the DNA in some manner. DNA-binding proteins or small molecules could be employed to shield the genetic material from water. In addition, an immense increase in DNA repair capacity could help counter the expected instability of a sugar-arsenate backbone. These types of cellular contingency plans seem likely to require the existence of specialized biochemical machinery that would simultaneously protect the DNA from hydrolysis while allowing normal readout and replication of the genetic code. At this point it may be important to reiterate the magnitude of the kinetic challenge that would face an organism with arsenodiester linkages in its DNA. The estimates presented in preceding paragraphs suggest that, if exposed to bulk water, half of the arsenodiester linkages in the genome of *Halomonadacea* GFAJ-1 would be hydrolytically cleaved in less than 0.1 s. While some bacteria have evolved mechanisms for protecting their DNA under conditions of stress,[36,37] overcoming such dramatic kinetic instability in its genetic material would be a significant feat for *Halomonadacea* GFAJ-1. Finally, we note that the use of arsenate esters in cell signaling, enzyme regulation, and cellular respiration would present a similar set of difficulties to the microbe.

In conclusion, the suggestion that *Halomonadacea* GFAJ-1 can utilize arsenic instead of phosphorus in its macromolecules and metabolites is certainly intriguing. With this Perspective, we do not mean to imply that such an organism *cannot* exist. Indeed, over the course of history, many things that were once unimaginable or thought to be implausible have gained widespread evidentiary support and come to be accepted. Rather, this Perspective serves to remind that quantitative consideration of chemical precedents can be important when sorting strong hypotheses from weak ones in biochemistry and biology. Considerations of the chemical and biochemical challenges faced by an arsenophilic microbe, thus far, have been superficial. In coming months and years, the scientific process will yield further evidence regarding whether *Halomonadacea* GFAJ-1 can or cannot function as an arsenophile, and careful considerations of chemical reactivity will be a central element of this story.

AUTHOR INFORMATION

Corresponding Author

*Phone: (573) 882-6763. Fax: (573) 882-2754. Email: gatesk@missouri.edu.

ACKNOWLEDGMENT

K.S.G. thanks Christina Wells for helpful discussions and Zachary Parsons for assistance in the preparation of Figure 1.

REFERENCES

(1) Westheimer, F. H. (1987) Why nature chose phosphates. *Science 235*, 1173–1178.

(2) Wolfe-Simon, F., Blum, J. S., Kulp, T. R., Gordon, G. W., Hoeft, S. E., Pett-Ridge, J., Stolz, J. F., Webb, S. M., Weber, P. K., Davies, P. C. W., Anbar, A. D., and Oremland, R. S. (2010) A bacterium that can grow by using arsenic instead of phosphorus, .*Science*; published online ahead of print,; DOI: 10.1126/science.1197258 .

(3) Wolfe-Simon, F., Davies, P. C. W., and Anbar, A. D. (2009) Did nature also choose arsenic? *Int. J. Astrobiol. 8*, 69–74.

(4) Huheey, J. E., Keiter, E. A. Keiter, R. L. (1993) *Principles of Structure and Reactivity*, 4th ed., Harper Collins, New York.

(5) Allred, A. L., and Hensley, A. L. J. (1961) Electronegativities of nitrogen, phosphorus, arsenic, antimony, and bismuth (trivalent states). *J. Inorg. Nucl. Chem. 17*, 43–54.

(6) Messens, J., and Silver, S. (2006) Arsenate reduction: thiol cascade chemistry with convergent evolution. *J. Mol. Biol. 362*, 1–17.

(7) Kerr, J. A. (2000) Bond enthalpies, in *CRC Handbook of Chemistry and Physics 1999−2000: A Ready-Reference Book of Chemical and Physical Data* (Lide, D. R., Ed.) CRC Press, Boca Raton.

(8) Schlegel, M. K., Essen, L.-O., and Meggers, E. (2010) Atomic resolution duplex structure of the simplified nucleic acid GNA. *JCS Chem. Comm. 46*, 1094–1096.

(9) Appella, D. H. (2009) Non-natural nucleic acids for synthetic biology. *Curr. Opin. Chem. Biol. 13*, 687–696.

(10) Moore, S. A., Moennich, D. M. C., and Gresser, M. J. (1983) Synthesis and hydrolysis of ADP-arsenate by beef heart submitochondrial particles. *J. Biol. Chem. 258*, 6266–6271.

(11) Doudoroff, M., Barker, H. A., and Hassid, W. Z. (1947) Studies with bacterial sucrose phosphorylase III. Arsenolytic decomposition of sucrose and of glucose-1-phosphate. *J. Biol. Chem. 170*, 147–150.

(12) Silver, S., Nucifora, G., Chu, L., and Misra, T. K. (1989) Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions. *Trends Biochem. Sci. 14*, 76–80.

(13) Purich, D. L. (2010) *Enzyme Kinetics: Catalysis and Control*, Academic Press, New York.

(14) Kline, P. C., and Schramm, V. L. (1993) Purine nucleoside phosphorylase. Catalytic mechanism and transition-state analysis of the arsenolysis reaction. *Biochemistry 32*, 13212–13219.

(15) Lewandowicz, A., and Schramm, V. L. (2004) Transition state analysis for human and *Plasmodium falciparum* purine nucleoside phosphorylases. *Biochemistry 43*, 1458–1468.

(16) Dickson, K. S., Burns, C. M., and Richardson, J. P. (2000) Determination of the free-energy change for repair of a DNA phosphodiester bond. *J. Biol. Chem. 275*, 15828–15831.

(17) Williams, N. H., and Wyman, P. (2001) Base catalysed phosphate diester hydrolysis. *Chem. Commun.* 1268–1269.
(18) Wolfenden, R., Ridgway, C., and Young, G. (1998) Spontaneous hydrolysis of ionized phosphate monoesters and diesters and the proficiencies of phosphatases and phosphodiesterases as catalysts. *J. Am. Chem. Soc. 120*, 833–834.
(19) Schroeder, G. K., Lad, C., Wyman, P., Williams, N. H., and Wolfenden, R. (2006) The time required for water attack at the phosphorus atom of simple phosphodiesters and of DNA. *Proc. Natl. Acad. Sci. U.S.A. 103*, 4052–4055.
(20) Fekry, M., I., and Gates, K. S. (2009) DNA-catalyzed hydrolysis of DNA phosphodiesters. *Nat. Chem. Biol. 5*, 710–711.
(21) Gates, K. S. (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. *Chem. Res. Toxicol. 22*, 1747–1760.
(22) Gates, K. S., Nooner, T., and Dutta, S. (2004) Biologically relevant chemical reactions of *N*7-alkyl-2′-deoxyguanosine adducts in DNA. *Chem. Res. Toxicol. 17*, 839–856.
(23) Eigner, J., Boedtker, H., and Michaels, G. (1961) The thermal degradation of nucleic acids. *Biochim. Biophys. Acta 51*, 165–168.
(24) Baer, C. D., Edwards, J. O., and Rieger, P. H. (1981) Kinetics of the hydrolysis of arsenate(V) triesters. *Inorg. Chem. 20*, 905–907.
(25) Ford, G. C., and Edwards, I. (1969) Tracer studies of the hydrolysis of arsenite, arsenate, and titanate esters. *Int. J. Mass Spectrom. Ion Phys. 2*, 95–97.
(26) Flam, F. (2010) Backing off arsenic eating claim, *Philadelphia Enquirer*. http://www.philly.com/inquirer/front_page/20101217_Backing_off_an_arsenic-eating_claim.html.
(27) Zimmer, C. (2010) "This paper should not have been published", In *Slate* (Plotzm D., Ed.). http://www.slate.com/id/2276919/.
(28) Begley, S. (2010) Arsenic and new life - or not: claims of a new life form get whacked, *Newsweek*. http://www.newsweek.com/2010/12/08/claims-of-a-new-life-form-get-whacked.html.
(29) Redfield, R. (2010) Arsenic-associated bacteria (NASA's claims), In *RRRESEARCH*. http://rrresearch.blogspot.com/2010/12/arsenic-associated-bacteria-nasas.html.
(30) Okumura, A., Yamamoto, N., and Okazaki, N. (1973) Kinetics of oxygen exchange between arsenate ions and water. II. Catalysis by arsenious acid. *Bull. Chem. Soc. Jpn. 46*, 3633–3635.
(31) Kok, B., and Varner, J. E. (1967) Extraterrestrial life detection based on oxygen isotope exchange reactions. *Science 155*, 1110–1112.
(32) Bhattacharjee, H., Sheng, J., Ajees, A. A., Mukhophadhyay, R., and Rosen, B. P. (2010) Adventitious arsenate reductase activity of the catalytic domain of the human Cdc25B and Cdc25C phosphatases. *Biochemistry 49*, 802–809.
(33) Thomas, D. J. (2010) Arsenolysis and thiol-dependent arsenate reduction. *Toxicol. Sci. 117*, 249–252.
(34) Waalkes, M. P., and Liu, J. (2002) Purine nucleoside phosphorylase: a fortuitous cytosolic arsenate reductase? *Toxicol. Sci. 70*, 1–3.
(35) Németi, B., Regonesi, M. E., Tortora, P., and Gregus, Z. (2010) Polynucleotide phosphorylase and mitochondrial ATP synthase mediate reduction of arsenate to the more toxic arsenite by forming arsenylated analogues of ADP and ATP. *Toxicol. Sci. 117*, 270–281.
(36) Setlow, P. (1995) Prevention of damage to DNA in spores of Bacillus species. *Annu. Rev. Microbiol.* 29–54.
(37) Grogan, D. W. (2004) Stabilty and repair of DNA in hyperthermic arachaea. *Curr. Opin. Cell. Biol. 6*, 137–144.