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Can Adenosine Triarsenate Role as an Energy Carrier?

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Recently, Wolfe-Simon *et al.* reported the existence of a bacterium species, GFAJ-1, which can grow using arsenic (As) instead of phosphorus (P).[1,2] If this is true, despite many objections that have risen,[3-5] it will revolutionize our knowledge of biochemistry and may even open a new door to the search for the existence of extraterrestrial life. However, before getting thrilled by this new discovery, we will have to investigate various aspects of many essential biomolecules when their P contents are replaced with As. In the case of DNA, which is definitely one of the core molecules in life, Denning *et al.*[6] and Mládek *et al.*[7] investigated the effect of the As-substitution and concluded that it induces no critical effect on the DNA backbone structure, although hydrolysis of this backbone will likely be quite rapid.[3,5]

One additional core biomolecule is adenosine triphosphate (ATP), which takes the role of an energy carrier of essentially all life forms. Many biochemical reactions are coupled with hydrolysis of ATP and phosphorylation, as ATP has high-energy phosphate bonds and releases a large amount of free energy ($\Delta G^{o} = -7.3$ kcal/mol[8]) upon the reaction. If As-based organism is possible, they will possess adenosine triarsenate (ATA) instead of ATP since there will be no or little P content to form ATP. Even forming the formerly discovered adenosine diphosphate arsenate (ADP-Asi)[2,9,10] will be unlikely under a harsh condition without any P in the environment. If the organism has adopted As as a brick of forming various biomolecules and/or as mortar for gluing them, ATA should (a) be structurally similar with ATP so that the relevant enzymes can adopt a similar mechanism as in the ATP case, (b) be stable enough to function regularly in biochemical systems, and (c) release a large enough amount of free energy during the hydrolysis reaction. Even though (a) has been shown to be feasible in a number of recent reports,[6,7] in the case of (b), there are many verified studies that argue arsenate ester is kinetically unstable in regard to hydrolysis.[3-5,11,12] In the present communication, we will show that the involved thermodynamics is also a great obstacle toward utilizing ATA or arsenate esters as energy carriers in biological systems.

In this work, the energetics of adenosine mono/di/triphosphates, A*n*P (*n* = M, D, T), and their As-substituents were computationally studied. The computational details are described in the Supporting Information. Ideally, for exact comparisons, we need to calculate free energy changes of the relevant reactions, which are computationally too demanding in many cases especially for quantum chemical approaches. However, because conformations of As-substituted compounds are close to the P-containing biological analogs,[6,7] we can assume that the contributions from the internal degrees of freedom toward the reaction entropies will be similar for both P and As-containing compounds. By additionally assuming that the solvent-related contributions will be mostly recovered from the implicit solvent model adopted here,[13] the changes in the reaction free energies ($\Delta\Delta G$) between ATP, ADP-Asi, and ATA hydrolysis reactions will be reasonably estimated from their reaction energy differences.

Table 1 shows the calculated energetics of hydrolysis reactions of ATP, creatine phosphate, and pyrophosphates together with their arsenate analogues. Hydrolysis energies of investigated phosphorylated compounds diminish by 4.06 –5.58 kcal/mol when P is substituted by As. This will lead to a severe loss in energy utilization from a thermodynamic point of view. Interestingly, recent density functional theory (DFT) results on activation energies of hydrolysis reaction indicate that methyl arsenate hydrolysis has much lower energy barrier than in the case of methyl phosphate ester (18.4 kcal/mol in arsenate ester versus 32.5 kcal/mol in phosphate ester).[3] It is clearly related to the instability of arsenate esters.[3,5,11,12] Thus, we can infer that adopting ATA will likely suffer from two disadvantages, both kinetically and thermodynamically. This situation can be pictorially

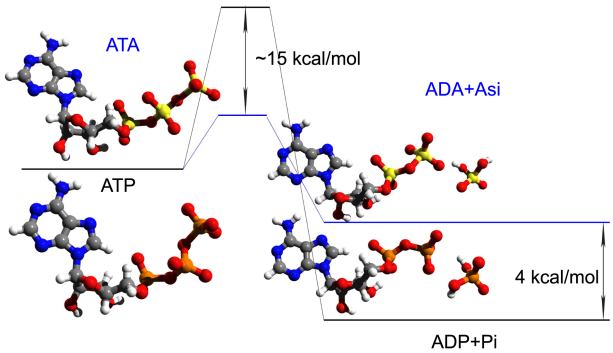

Figure 1. Cartoon representation of free energy profiles of ATP and ATA hydrolysis. Overall, arsenylation imposes disadvantages in terms of both kinetics and thermodynamics: instability against unwanted hydrolysis (see refs. 3-5, 11, 12) and loss of efficiency in energy generation.

Table 1. Hydrolysis energy changes ($\Delta\Delta E$)[a] in kcal/mol by arsenic substitutions of ATP, creatine phosphate, and pyrophosphate, calculated at the RI-MP2/aug-cc-pVTZ level with COSMO solvent model

ATP Hydrolysis[e]				
	Overall	HF[b]	Correlation[c]	Solvation[d]
ATP	0.000	0.000	0.000	0.000
ADP-Asi	2.888	9.517	1.645	−8.274
ATA	4.061	13.815	3.051	−12.805
Creatine phosphate hydrolysis[e]				
	Overall	HF[b]	Correlation[c]	Solvation[d]
cPi	0.000	0.000	0.000	0.000
cAsi	5.581	7.532	1.899	−3.850
Pyrophosphate hydrolysis[e]				
	Overall	HF[b]	Correlation[c]	Solvation[d]
Pi-Pi	0.000	0.000	0.000	0.000
Asi-Pi	2.453	5.593	1.487	−4.627
Asi-Asi	4.785	12.458	2.915	−10.588

[a]Reaction energy changes, $\Delta\Delta E$, are measured as ΔE (target reaction) – ΔE (all phosphorus reaction). [b]Hartree-Fock contribution to the overall reaction energy. [c]RI-MP2 electron correlation contribution to the overall reaction energy. [d]COSMO solvation contribution to the overall reaction energy. [e]Schemes for these reactions are in Table S2 in the Supporting Information.

represented with phosphate/arsenate hydrolysis energetics as shown in Figure 1. From the experimental value of hydrolysis free energy of ATP in the standard state (−7.3 kcal/mol), the hydrolysis free energy of ATA is estimated to be −3.2 kcal/mol. This will likely be insufficient to turn the arsenate compounds to role as effective energy carriers in biological system. For example, during the glycolysis from glucose to pyruvate, where two molecules of ATP (−14.6 kcal/mol of energy content) are generated, only −6.4 kcal/mol of free energy will become available with ATA. Such a loss will occur during all metabolic processes and will cause a great loss of energetic efficiency. More importantly, this difference will shift the equilibrium positions of various biological reactions coupled with ATP hydrolysis as will be discussed further in the Supporting Information.

By calculating energetics of P-containing and As-containing compounds using different basis sets, we concluded that the basis the set superposition error is not the origin of the energetic differences (see Supporting Information). To investigate the actual origin of the difference, we have considered the energies of the hydrolysis reactions in three separate components-Hartree-Fock (HF), RI-MP2 correlation energy,[14-16] and COSMO solvation energy.[13] The results of these decompositions are shown in Table 1. Solvation actually stabilizes the products from the hydrolysis of ATA and other As-substituted compounds. However, this is more than compensated by HF energy components. In fact, the HF component mostly represents the direct chemical bonding energy. Thus, one can infer that the energetic cost for breaking the arsenate bond is larger than the "high-energy" phosphate bond. The contribution of electron correlation is quite smaller than other contributions, but follows the same trend as the HF components. Overall, the energy contents in various arsenate bonds are thermodynamically not as high as in the corresponding phosphate bonds, at least in regard to hydrolysis reactions.

In conclusion, our results suggest that arsenic analogs of ATP or related phosphorylated compounds are inappropriate as energy carriers as they will release much smaller free energies for various biochemical reactions. This will shift the balances of essential biochemical equilibria. Even if environment in biological system such as enzymatic protein structure is optimized to stabilize arsenate esters, the hydrolysis energies of the As-analogs will likely limit their role as energy carriers. When this aspect is considered in combination with the already reported kinetic instability of arsenate esters and As-DNA,[3,5,11,12] it is unlikely that As-substituted analogs will be functional as replacements of conventional phosphorylated adenosine compounds. If As-based life should exist, they will have to survive with a new mechanism and/or different chemical species other than ATA as an energy carrier.

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Supporting Information. Computational details, equilibrium shifts in various ATP-coupled reactions, RI-MP2/aug-cc-pVDZ version of hydrolysis energy changes ($\Delta\Delta E$) in kcal/mol for the same reactions shown in Table 1 together with schemes.

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