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ORIGINAL ARTICLE

Potassium-encapsulated arsenic-dithiolato compounds: Synthesis, structural calculation, and biological relevance

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Abstract This study based on the synthesis, characterization, and structural calculation of small molecular potassium-encapsulated arsenic-dithiolato compounds will provide fundamental knowledge about arsenic metabolism behavior in biological system. Two novel air-stable potassium-encapsulated arsenic-dithiolato compounds, $[K@As_2(L1)_3](BF_4)$ (**1**) and $[K@As_2(L2)_3](BF_4)$ (**2**), were prepared using deprotonated 2,6-bis(mercaptomethyl)pyridine ($L1H_2$) and 1,3-dimercapto-*m*-xylene ($L2H_2$) to react with $AsCl_3$ in the presence of potassium cation. Compounds **1** and **2** have been characterized by electrospray ionization-mass spectra, nuclear magnetic resonance spectra, and elemental microanalysis. Density functional theory calculation also supports the formation and binding properties of the potassium-encapsulated arsenic-dithiolato compounds.

Introduction

Arsenic is one of the most toxic inorganic ions. It is also a natural contaminant of water supplies in global environmental problem because arsenic can enter groundwater systems from weathering and leaching of arsenic minerals in rock and soil [1,2]. Compounds of arsenic found in the environment are broadly classified as either organic or inorganic with two common oxidation states of 3+ and 5+. Studies on the toxicity of arsenic ion shows that there are two forms of arsenic: one is arsenate (As^V; $HAsO_4^{2-}$) and the other is arsenite [As^{III}; $As(OH)_3$] [3]. When arsenate is imported into cells by the anion transport pathway, it disrupts cellular processes by mimicking as phosphate. The arsenate may reduce to arsenite by thiol-rich proteins within cells, which is shown by numerous literature studies [4]. Usually, proteins undergo a critical conformational change on binding As^{III} with two or three cysteine residues

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[5,6]. The arsenite, forming strong As—S bond with cysteine thiol residues, which may form a three-coordination As^{III} compound [7—10], is more toxic than arsenate and has been shown to disrupt the activity of certain enzymes, such as pyruvate dehydrogenase [11,12], glutathione reductase [13], and thioredoxin reductase [14].

From the chemistry point of view, the monothiols bind arsenicals rather weakly, although the relatively high intracellular concentration of free glutathione will strongly influence arsenic speciation in mammalian cells. In contrast, protein dithiols bind monoalkyl or monoaryl As^{III} species much more tightly by means of the chelating effect [9,15]. Indeed, many of the suggested protein targets of arsenicals have dithiols or trithiols in close proximity [16—19]. Despite arsenic's biological precedence and implications on health, there is relatively little structural detail known about its interactions with proteins and also still remains a general lack of small-molecule As^{III} compounds in biological coordination environments [20—25]. In this study, we synthesized two types of dithiolate ligands 2,6-bis(mercaptomethyl)pyridine ($L1H_2$) and 1,3-dimercapto-*m*-xylene ($L2H_2$) to imitate the protein cysteine residues that react with the arsenic ion as structural biomimicking models. These studies on the synthesis and characterization of simple potassium-encapsulated arsenic-dithiolato compounds will provide fundamental knowledge about arsenic metabolism behavior in biological system.

Materials and methods

General considerations

All manipulations were carried out in an atmosphere of purified dinitrogen with standard Schlenk techniques. Chemical reagents were purchased from Aldrich Chemical Company Ltd., Lancaster Chemicals Ltd., or Fluka Ltd. All the reagents were used without further purification, apart from all solvents that were dried over Na (Et_2O, tetrahydrofuran; THF) or CaH_2 (CH_2Cl_2, CH_3CN) and then thoroughly degassed before use. 2,6-Bis(mercaptomethyl) pyridine ($L1H_2$) [26] and 1,3-dimercapto-*m*-xylene ($L2H_2$) [27] were prepared according to the literature procedures. ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were acquired on a Varian Gemini-200 proton/Carbon FT NMR or a Varian Gemini-500 proton/Carbon FT NMR spectrometer. Electrospray ionization (ESI)-mass spectra were collected on a Waters ZQ 4000 mass spectrometer. Elemental analyses were performed on a Heraeus CHN-OS Rapid Elemental Analyzer.

Synthesis of $[K@As_2(L1)_3](BF_4)$ (1)

2,6-Bis(mercaptomethyl)pyridine ($L1H_2$; 0.70 mmol, 120 mg) was added to a solution of THF (50 mL) and KOH (2.14 mmol, 120 mg) in CH_3OH (20 mL). This solution was heated to 50°C, and $AsCl_3$ (0.50 mmol, 90 mg) was added dropwise. The reaction mixture was stirred at room temperature for 3 hours and dried *in vacuo* to give a white solid. The white solid was extracted by CH_3CN (15 mL × 3), and then, KBF_4 (0.24 mmol, 30 mg) in CH_3CN (10 mL) was added into the extract to give a white precipitate. The resulting white precipitate was removed by filtration, and the filtrate was dried *in vacuo* (yield, 136 mg; 75%). ^1H NMR spectra (DMSO)-d_6): δ 3.70 (s, 4H, Py-CH_2-S); 7.01 (d, 2H, Py-*H*); 7.49 (t, 1H, Py-*H*). $^{13}C\{^1H\}$ NMR (DMSO-d_6): δ 38.63 (Py-*C*H_2-S), 120.53 (Py), 136.63 (Py), 161.66 (Py). ESI-mass spectra: 695.70 $[K@As_2(C_7H_7NS_2)_3]^+$. Anal. Calcd. for $C_{21}H_{21}As_2BF_4KN_3S_6$: C, 32.19; H, 2.70; N, 5.36. Found: C, 32.22; H, 2.72; N, 5.32.

Synthesis of $[K@As_2(L2)_3](BF_4)$ (2)

1,3-Dimercapto-*m*-xylene ($L2H_2$; 0.66 mmol, 112 mg) was added to a solution of THF (50 mL) and KOH (1.96 mmol, 110 mg) in CH_3OH (15 mL). This solution was heated to 50°C, and $AsCl_3$ (0.44 mmol, 80 mg) was added dropwise. The reaction mixture was stirred at room temperature for 3 hours and dried *in vacuo* to give a white solid. The white solid was extracted by CH_3CN (15 mL × 3), and then, KBF_4 (0.24 mmol, 30 mg) in CH_3CN (10 mL) was added into the extract to give a white precipitate. The resulting white precipitate was removed by filtration, and the filtrate was dried *in vacuo* (yield, 108 mg; 63%). ^1H NMR spectra (DMSO-d_6): δ 3.82 (s, 4H, Ph-CH_2-S); 7.10 (s, 1H, Ph-*H*); 7.21 (d, 2H, Ph-*H*); 7.43 (t, 1H, Ph-*H*). $^{13}C\{^1H\}$ NMR spectra (DMSO-d_6): δ 25.43 (Ph-*C*H_2-S), 126.15 (Ph), 128.73 (Ph), 130.05 (Ph), 139.66 (Ph). ESI-mass spectra: 692.71 $[K@As_2(C_8H_8S_2)_3]^+$. Anal. Calcd. for $C_{24}H_{24}As_2BF_4KS_6$: C, 36.93; H, 3.10. Found: C, 37.01; H, 3.15.

Computational methods

Density functional theory (DFT) calculations of B3LYP/LANL2DZ level were used to provide the information regarding the structure, binding energy, and charge distribution of Compounds **1** and **2**. Geometry optimizations with C_{3h} constraint were carried out for compounds containing potassium cation, whereas no symmetric constraint (i.e. C_1 symmetry) was imposed to geometry optimization calculations for complexes without potassium cation. Frequency calculations were performed to confirm that all the optimized structures were indeed a local minimum on the potential energy surface. The charge distributions were analyzed by using the Mulliken population analysis. The binding energy of K^+ was estimated by the energetic difference between the total energy of potassium-contained complex and that of free complex and K^+. All calculations were accomplished by Gaussian 09 program [28].

Results and discussion

The dithiolate ligands 2,6-bis(mercaptomethyl)pyridine ($L1H_2$) and 1,3-dimercapto-*m*-xylene ($L2H_2$) are synthesized by literature procedure [26,27]. When a pale yellow THF solution of a $L1^{2-}$ or $L2^{2-}$ (deprotonated with 2 mol equiv. of sodium methyloxide) was treated with 0.33 mol equiv. of $AsCl_3$, an insoluble yellow precipitate immediately formed, which is proposed to be a polymer of formula As_2L_3 (L = $L1^{2-}$ or $L2^{2-}$); this was proved by elemental analyses. However, if potassium salt agents (potassium methyloxide or potassium hydride) are used in the deprotonation process, a potassium-encapsulated cationic

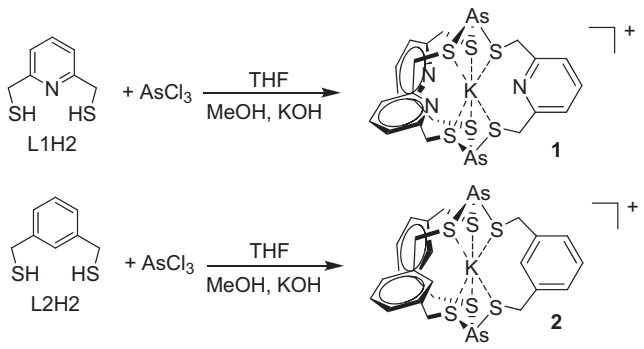

Figure 1. Representation synthesis of Compounds **1** and **2**.

diarsenic compound forms having the formula $[K@As_2(L)_3]^+$ [L = $L1^{2-}$ (**1**); $L2^{2-}$ (**2**)], which can be confirmed by ESI-mass technique. ESI is a very mild ionization technique with minimum perturbations of the species presented in solution and have been performed on the monitoring reaction processes [29—31].

To give more information about the formation of cationic dimer compounds $[K@As_2(L1)_3]^+$ (**1**) and $[K@As_2(L2)_3]^+$ (**2**) in presence of potassium cation, we also examined the reaction of dithiol ligands ($L1H_2$ or $L2H_2$) with stoichiometric amounts of $AsCl_3$ and KOH in THF/methanol mixtures (Fig. 1). In the KOH assistance, cationic products **1** and **2** are formed and characterized by ESI-mass spectra. Both cationic compounds **1** and **2** contain three dithiolato ligands and incorporate with one potassium cation, which can be confirmed by their formula by well-resolved ESI-mass spectra with the pattern of molecular ion peaks (Fig. 2), respectively. On the other hand, NaOH is a substitute for KOH during the reaction; however, only insoluble yellow precipitates present. This indicates that only potassium can assist cationic diarsenic compounds **1** and **2** formation. Additionally, the very simple ^1H and ^{13}C NMR data of **1** and **2** also suggest the high-symmetry C_{3h} structure and the absence of any rapid equilibration between the dithiolato chelate rings. This profile indicates the molecular structure of the cation core of **1** and **2**, because the nature of chelation to potassium cation and As^{III} atom results in the ligands being equivalent to each other.

When methanol solutions of **1** or **2** are purged with air or O_2(g) for 5 minutes, no significant change in the ESI-mass and ^1H NMR spectra are observed over 1 hour. From this observation, compounds **1** and **2** demonstrate remarkable stability under aerobic conditions, suggesting that these potassium cation-assistance self-assembled compounds are not only kinetic products but also, indeed, the thermodynamic products of the reaction between arsenic and dithiolato ligands. There was no ligand exchange between **1** and $L2^{2-}$ ligand on the ESI-mass monitoring and vice versa. Compounds **1** and **2** also were robust to a variety of competing metal ions, such as Na(I), Ag(I), Cu(I), Fe(II), and Zn(II).

Hsu and Boyer et al. have shown that alkali cation—templated cage formation is controlled by the radius of

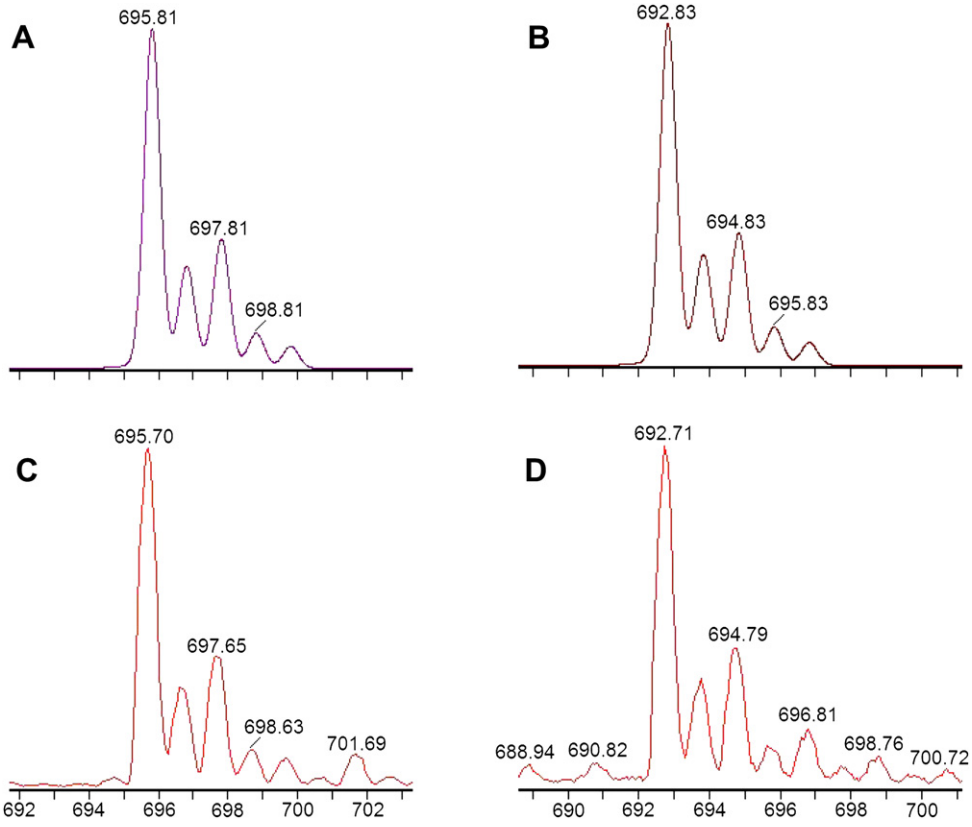

Figure 2. Calculated (A and B) and experimental (C and D) electrospray ionization-mass spectrum of the potassium-encapsulated cationic diarsenic compounds **1** (A and C) and **2** (B and D).

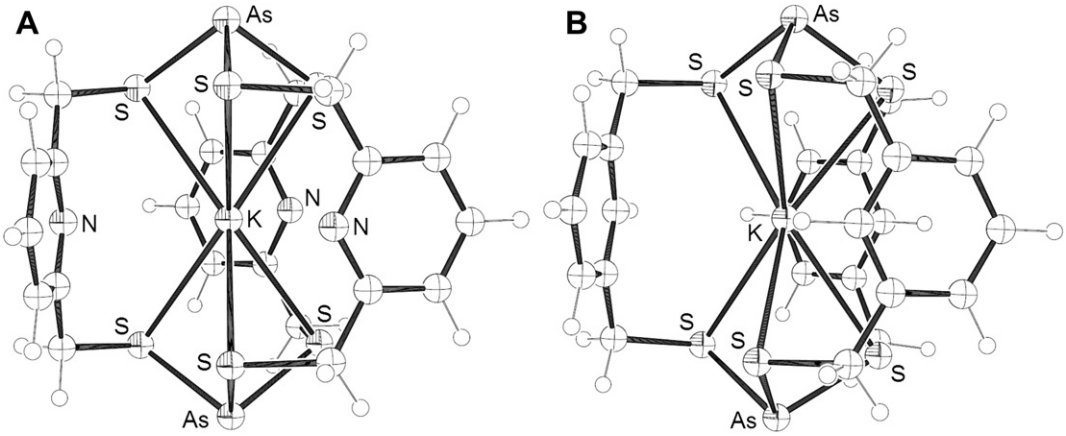

Figure 3. Molecular structure of the cations of compounds **1** (A) and **2** (B) in density functional theory calculation results at the 50% level.

alkali metal cation [30,32]. The potassium cation would serve as an essential template for a larger cage compared with the sodium cation for small cage formation. In our case, the formation of potassium-encapsulated cationic diarsenic compounds **1** and **2** may be assisted and controlled by the size of templated cation. To provide insights into the properties of compounds **1** and **2**, DFT of B3LYP/LANL2DZ method was used to locate the optimized structures and to evaluate the binding energies of dithiolato ligands with K^+. The DFT geometry optimizations revealed several interesting structural features for compounds **1** and **2** (Figs. 3 and 4). Both compounds have similar structural features and are characterized by a cage structure formed by the three dithiolato ligands and two arsenicals through S—As interactions with a potassium cation located in the center of the cage. The arsenicals are situated outward the cage and, therefore, have no contribution to the binding with potassium cation. Instead, the embedded potassium cation is stabilized by six S—K^+ interactions. Furthermore, the aromatic rings of the ligands use their face rather than edge pointing toward the interior of the cage; for compound **1**, the nitrogen atom of pyridine ring is nearest to K^+ with a distance of 3.21 Å, whereas for compound **2**, the shortest contact between benzene ring and K^+ is 3.40 Å (Table 1). It is worth to notice that the cavity size of compound **2** is somewhat larger than that of compound **1**, which can be evidenced from the longer distances of S—K^+ and ring—K^+ contacts in the former than in the latter (Table 1). The effect of cavity size truly reflects on the binding ability to potassium cation; the theoretical estimate of binding energy of K^+ is −53.7 kcal/mol for compound **1**, remarkably greater than −45.6 kcal/mole for compound **2**. The Mulliken population analysis indicated that the charge—transfer interaction should play a role in stabilizing the potassium cation; note that K^+ bears only 0.67 |e| positive charge (Table 2); in other words, about 0.33 |e| negative charge is transferred from the ligands to potassium.

Herein, we have synthesized and characterized the potassium-encapsulated cationic diarsenic compounds **1** and **2**. Further investigation of DFT calculation shows that potassium-encapsulation ability is affected by the cavity size of arsenic-dithiolato-based capsules. It is well known

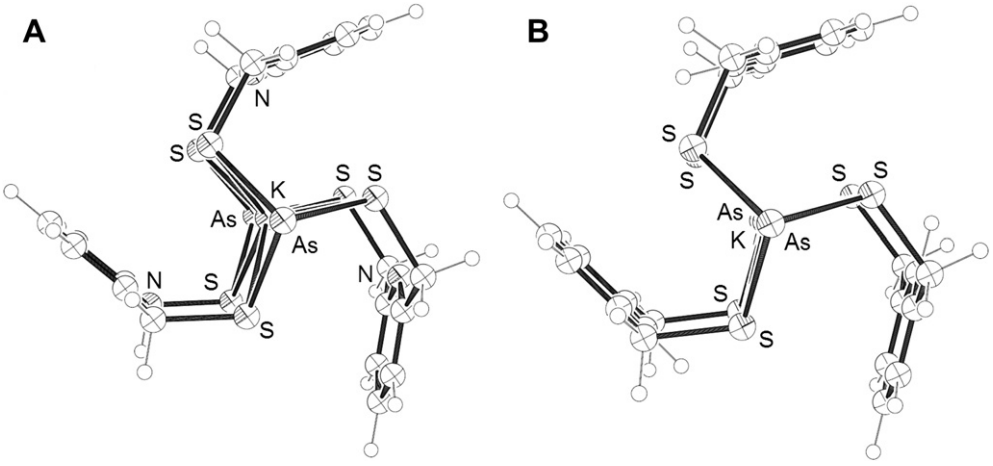

Figure 4. Pseudo-threefold views of the cations of compounds **1** (A) and **2** (B) in density functional theory calculation results at the 50% level.

Table 1 Selected B3LYP/LANL2DZ optimized distances (Å) of compounds **1** and **2**

Compound	Distance (Å)
1	
S to As	2.44
S to K	3.31
N to K	3.21
As to As	7.72
2	
S to As	2.44
S to K	3.39
C to K	3.40
As to As	7.84

Table 2 Mulliken atomic charges for compounds **1** and **2**

	Compound **1**	Compound **2**
As	0.420	0.453
K	0.673	0.675
S	−0.142	−0.179

that trivalent arsenic can bind to cysteine residues of proteins [4,33]. $L1^{2-}$ and $L2^{2-}$ display simultaneous SNS and SS interaction for cysteine and histidine units of protein, which may interact with As^{III} and encapsulate potassium cation in biological system. There are not many good biologically relevant models of arsenic compounds in biological coordination environments, it is the first time that potassium-encapsulation behavior by the chelated dithiolate ligands cooperated with arsenic system is observed; this finding may provide fundamental clue to construct As—base chemosensors, which is in progress in studies in our laboratory.

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