Journal of Hazardous Materials 280 (2014) 734–740

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Kinetics and activation parameters of the reaction of organoarsenic(V) compounds with glutathione

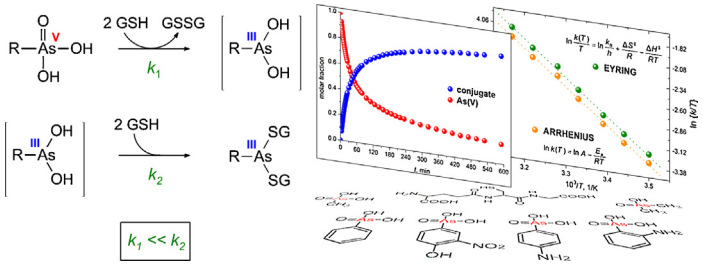
Jerome Kretzschmar[a,*,1], Erica Brendler[a], Jörg Wagler[b], Anne-Christine Schmidt[a]

[a] Institute of Analytical Chemistry, TU Bergakademie Freiberg, 09596 Freiberg, Germany
[b] Institute of Inorganic Chemistry, TU Bergakademie Freiberg, 09596 Freiberg, Germany

HIGHLIGHTS

- The redox reaction of organoarsenic(V) and GSH is the rate determining step.
- All investigated arylarsenicals are subject to the same reaction mechanism.
- Arylarsenicals(V) react faster with GSH than methylated arsenicals(V).
- Arylarsenicals(V) show correlation between kinetics and toxicity.
- The kinetics explain the higher toxicity of As(III) compared to As(V).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 12 July 2014
Accepted 12 August 2014
Available online 28 August 2014

Keywords:
GSH
Roxarsone
Phenylarsonic acid
Arsenic detoxification
Kinetics
NMR

ABSTRACT

In this work the kinetics of the reaction of glutathione (GSH) with the organoarsenic(V) compounds phenylarsonic acid (PAA), 4-hydroxy-3-nitrophenylarsonic acid (HNPAA), *p*-aminophenylarsonic acid (*p*-APAA) and *o*-aminophenylarsonic acid (*o*-APAA) as well as monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) is investigated. The reaction progress is monitored in real time by ^{1}H NMR, allowing the determination of rate coefficients and half-lives as well as activation parameters. The reaction consists of two steps: redox reaction and conjugation. In all investigated systems the conjugation is fast compared to the redox reaction and, therefore, rate determining. All investigated phenylarsonic acids follow the same rate law, showing overall reaction orders of 3 and half-lives between 47.7 ± 0.2 and 71.0 ± 3.6 min. The methylated compounds react slower, showing half-lives of 76.6 ± 0.4 and 444 ± 10 min for DMAA and MMAA, respectively. Enthalpies of activation range from 20 to 36 (±2) kJ mol^{-1} and the entropies of activation are within −154 and −97(±7) J mol^{-1} K^{-1}. The results reveal a correlation of the toxicity of the arsenic compound and the reaction rate with GSH. This may pave the way for the estimation of the toxicity of such compounds by simple kinetic studies.

Abbreviations: GSH, glutathione; PAA, phenylarsonic acid; HNPAA, 4-hydroxy-3-nitrophenylarsonic acid; *p*-APAA, *p*-aminophenylarsonic acid; *o*-APAA, *o*-aminophenylarsonic acid; MMAA, monomethylarsonic acid; DMAA, dimethylarsinic acid; GSSG, glutathione disulfide; pH*, deuterium-uncorrected pH; COSY, correlated spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation; TSP, 3-(trimethylsilyl)-propionic acid sodium salt; δ, chemical shift; GS, glutathionyl residue; *J*, scalar coupling constant; vic, vicinal; gem, geminal; *n*, overall reaction order; *k*, rate coefficient; *r*, observed reaction rate; $E_{1/2}$, half-cell potential.

* Corresponding author. Tel.: +49 351 260 3154; fax: +49 351 260 13233.
E-mail addresses: j.kretzschmar@hzdr.de (J. Kretzschmar), Erica.Brendler@chemie.tu-freiberg.de (E. Brendler), joerg.wagler@chemie.tu-freiberg.de (J. Wagler), chris_acs1@gmx.de (A.-C. Schmidt).
[1] Current address: Institute of Resource Ecology, Helmholtz-Zentrum Dresden-Rossendorf, P.O. Box 510119, 01314 Dresden, Germany.

http://dx.doi.org/10.1016/j.jhazmat.2014.08.036
0304-3894/

1. Introduction

Organoarsenic compounds play a crucial role in environmental contaminations caused by residues of arsenic containing chemical warfare agents [1–3]. Namely diphenylarsine chloride and cyanide, phenarsazine chloride, and phenylarsine dichloride were produced in large scale of several thousand tons during the First and Second World War [4]. At former military sites hydrolysis and oxidation products contaminate soils and waters resulting in high arsenic levels [5]. Some phenylarsenic compounds, especially 4-hydroxy-3-nitrophenylarsonic acid (HNPAA, roxarsone) and *p*-aminophenylarsonic acid (*p*-APAA) have been used as growth promoters and veterinary drugs in poultry and swine feed additives [6–10]. Arsenic-contaminated poultry litter is deposited or used as fertilizer in crop fields, thus leading to elevated arsenic concentrations in soils and leaching water [11–14]. While roxarsone is excreted unaltered in chicken manure (dominating the arsenic speciation in fresh poultry litter), it is microbiologically degraded during composting [11,15], producing a variety of organic and inorganic arsenicals [16–18]. Methylated arsenic compounds, including monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) also occur as metabolites from microbiological degradation [19] or from intracellular detoxification processes as found in human urine [20,21].

Glutathione (GSH) as a highly concentrated intracellular reducing agent plays a major role in various detoxification mechanisms of electrophiles such as radical oxygen species, heavy metals or metalloids [22–24]. This thiol functionalized tripeptide is particularly suitable for binding arsenic and due to its small size useful as a model molecule. Even in artificial systems consisting of the arsenic compound and GSH only, the reaction between these compounds occurs spontaneously. By reacting arsenic(V) compounds and GSH, arsenic is reduced to arsenic(III) by GSH while the latter is oxidized to glutathione disulfide (GSSG). Then, a covalent bond is formed between the arsenic(III) compound and the GSH sulfur [25] as shown in Eqs. (1a) and (1b) and proven by mass spectrometry [26] and NMR spectroscopy:

$$As^{(V)}O(OH)_{3-x}R_x + 2GSH \rightarrow As^{(III)}(OH)_{3-x}R_x + GSSG + H_2O \quad (1a)$$

$$As^{(III)}(OH)_{3-x}R_x + 3 - xGSH \rightarrow As^{(III)}(SG)_{3-x}R_x + 3 - xH_2O \quad (1b)$$

where $x = 2$ for DMAA, $x = 1$ for the other investigated compounds. R denotes the respective organic residues (cf. Fig. 1).

In the last decades, the chemical reaction of GSH with several arsenic compounds was repeatedly investigated [27,28], but respective kinetic data are still missing. Solely Ngu and Stillman [29] determined them for the reaction of inorganic arsenic with human metallothionein, applying mass spectrometry.

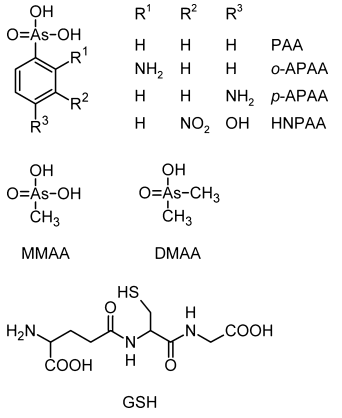


Fig. 1. Lewis structures of the investigated reactants.

Although all investigated arsenicals are toxic, their toxicity varies significantly. Since we could monitor their reaction with GSH by NMR and observed strong differences in the reaction rate, the quest arose whether there is a correlation between the kinetics of the reaction of GSH with environmental arsenic contaminants and their toxicity. Thus, in addition to a structural elucidation, the determination of characteristic kinetic parameters became a further fundamental motivation of this work. This involved reaction order, rate coefficient and half-life of the reaction of GSH with phenylarsonic acid (PAS), HNPAA, *p*-APAA and *o*-aminophenylarsonic acid (*o*-APAA) as well as MMAA and DMAA. Furthermore, the reaction was monitored at variable temperatures to obtain enthalpy, entropy and Gibbs free energy of activation.

2. Materials and methods

2.1. Materials

All arsenic containing compounds used were of analytical grade and purchased from Acros Organics, except DMAA, which was delivered by Greyhound, Birkenhead, UK. Glutathione (>99%, Sigma-Aldrich) was used as obtained without further purification to prepare stock solutions in deuterium oxide (D_2O, 99.98%, Armar Chemicals). The pH* (deuterium-uncorrected pH) was adjusted by addition of aliquots of a 50 mM deuterochloric acid (DCl) stock solution made from concentrated DCl (35 wt.% in D_2O, 99 at.% D, Sigma-Aldrich) in D_2O.

2.2. NMR spectroscopy

All experiments were performed on a Bruker Avance III 500 spectrometer with a magnetic field of 11.75 T corresponding to resonance frequencies of 500.13 MHz and 125.76 MHz for ^1H and ^{13}C, respectively, using a 5 mm indirect detection probe with z-gradient. An acquisition time of 5 s, 8 scans and a relaxation delay of 5 s were applied, monitoring the reaction until the conversion exceeded 70%. For structure elucidation, 2D-NMR, i.e. H,H-COSY, H,C-HSQC and H,C-HMBC spectra were recorded. All spectra were referenced relative to 3-(trimethylsilyl)-propionic acid sodium salt (TSP, 98%, Sigma-Aldrich) with $\delta_H = 0$ ppm and $\delta_C = 0$ ppm. Temperature stability (±0.1 K) was achieved by the spectrometer's temperature control combined with a BCU05 cooling unit (Bruker).

2.3. Kinetic study

The samples were degassed to prevent autoxidation by dissolved oxygen and used immediately after preparation. Reaction samples were prepared by diluting appropriate volumes of stock solutions to obtain 20 mM of GSH and 5 mM of arsenic components, except for DMAA, which was 6.67 mM. These molar ratios correspond to the stoichiometry of the reaction of GSH with the respective arsenicals. All measurements were carried out at 22 ± 0.2 °C. For the comparative investigation of all organoarsenic compounds a pH* of 3.0 ± 0.1 was chosen. By mixing GSH and the arsenic compound, the reaction and the time measurement were started. For preliminary investigations to assign the NMR signals the samples were prepared in the same way but allowed to react for at least one day protected from oxygen.

2.4. Activation parameters

Preparation and measurement were similar to that for the kinetic study (see Section 2.3), but carried out at temperatures between 10 and 40 °C with intervals of 5 K. Each measurement was repeated at least twice.