Biochimie 106 (2014) 167–174

Contents lists available at ScienceDirect

Biochimie

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

Research paper

Inhibitory mechanism of dimercaptopropanesulfonic acid (DMPS) in the cellular biomethylation of arsenic

Shuping Wang [a], Nan Shi [a], Zhirong Geng [a], Xiangli Li [a], Xin Hu [b], Zhilin Wang [a, *]

[a] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, PR China
[b] The Modern Analysis Center, Nanjing University, PR China

ARTICLE INFO

Article history:
Received 31 May 2014
Accepted 27 August 2014
Available online 4 September 2014

Keywords:
Arsenic
Inhibition
Biomethylation
DMPS
Mechanism

ABSTRACT

Dimercaptopropanesulfonic acid (DMPS) has been approved for the treatment of arsenic poisoning through promoting arsenic excretion and modulating arsenic species. To clarify how DMPS regulates the excretion of arsenic species, we investigated the effects of DMPS on the biomethylation of arsenite (As^{3+}) in HepG2 cells. In the experiments, we found that DMPS at low concentrations dramatically decreased the content of arsenic in HepG2 cells and inhibited the cellular methylation of As^{3+}. Three aspects, the expression of human arsenic (III) methyltransferase (hAS3MT), the accumulation of cellular reactive oxygen species (ROS) and the *in vitro* enzymatic methylation of arsenic, were considered to explain the reasons for the inhibition of DMPS in arsenic metabolism. The results suggested that DMPS competitively coordinated with As^{3+} and monomethylarsonous acid (MMA^{3+}) to inhibit the up-regulation of arsenic on the expression of hAS3MT and block arsenic involving in the enzymatic methylation. Moreover, DMPS eliminated arsenic-induced accumulation of ROS, which might contribute to the antidotal effects of DMPS on arsenic posing.

1. Introduction

As an ancient drug used in traditional Chinese medicine, arsenic shows anticancer activity [1–4]. Since arsenic trioxide has been successfully used in the treatment of acute promyelocytic leukemia, the anticancer efficacy of arsenic attracted increasing attentions [5]. However, arsenic, a significant hazard, is associated with many types of diseases including cancers [6,7]. The injury of arsenic on organs, especially liver and kidney, limits its application in the treatment of cancers [6,8]. The toxicity of arsenic is closely related to its metabolism in human body [7]. Hitherto, researches on arsenic toxicity suggested that the biomethylation of arsenic deepened its bio-toxicity [9].

In mammals, arsenic (III) methyltransferase catalyzed methylation is the major biotransformation of arsenic [10–12]. Up to now, oxidative methylation and glutathione (GSH) conjugation have been proposed to clarify the mechanism of arsenite (As^{3+}) methylation [13–15]. Although the mechanism of cellular As^{3+} methylation is unclear, peoples have found that a variety of reactive oxygen species (ROS) were generated during the pathway [16,17]. Hayakawa et al. regarded that As^{3+} was gradually methylated to monomethylarsonous acid (MMA^{3+}) and dimethylarsinous acid (DMA^{3+}) and then oxidized to their pentavalent forms [15]. Besides As^{3+}, MMA^{3+} and DMA^{3+} easily reacted with sulfhydryl groups to inactive many enzymes [9]. Toxicological studies showed that cytotoxic and genotoxic of MMA^{3+} and DMA^{3+} were higher than As^{3+} [7,9]. Above all, the toxicity of arsenic is related to the biomethylation itself and the trivalent metabolites.

Dimercaptopropanesulfonic acid (DMPS), dimercaptosuccinic and dimercaptopropanol were generally used in the treatment of arsenic poisoning for their powerful chelation [18]. Compared with the other two, the water-soluble DMPS showed higher therapeutic index and lower acute toxicity [18,19]. Clinical trials confirmed that DMPS increased the urinary excretion of total arsenic and monomethylated arsenicals (MMAs), but decreased the excretion of

Abbreviations: DMPS, dimercaptopropanesulfonic acid; As^{3+}, arsenite; As^{5+}, pentavalent arsenic; MMA^{3+}, monomethylarsonous acid; DMA^{3+}, dimethylarsinous acid; MMA^{5+}, monomethylarsonic acid; DMA^{5+}, dimethylarsinic acid; MMAs, monomethylated arsenicals; DMAs, dimethylated arsenicals; PBS, phosphate buffer; GSH, glutathione; AdoMet, S-Adenosyl-L-methionine; hAS3MT, human arsenic (III) methyltransferase; ROS, reactive oxygen species; HPLC, high performance liquid chromatography; ICP, inductively coupled plasma; MS, mass spectrometry; DCFH-DA, 2′,7′-dichlorodihydrofluorescein-diacetate; DCF, 2′,7′-dichlorofluorescin; DMEM, Dulbecco's Modified Eagle Medium; PI, propidium iodide.

* Corresponding author. Tel.: +86 25 8368 6082; fax: +86 25 8331 7761.
E-mail address: wangzl@nju.edu.cn (Z. Wang).

http://dx.doi.org/10.1016/j.biochi.2014.08.017
0300-9084/

dimethylated arsenicals (DMAs) in acute arsenic poisoning [19,20]. Aposhian et al. proposed that DMPS completely coordinated with MMA^{3+} in the form of DMPS-MMA^{3+}, which was not active substrate for the MMA^{3+} methylation and readily excreted in the urine [21,22]. Meanwhile, Heinrich-Ramm et al. suggested that DMPS might inhibit the second step methylation from MMA^{3+} to DMA^{3+} through analyzing the arsenic species in urine [19]. However, it is far from enough to clarify the reasons for arsenic detoxification through analyzing the arsenic species in urine.

To gain insight into the mechanism for DMPS detoxification, we studied the effects of DMPS on the biomethylation of As^{3+} in HepG2 cells. We found that DMPS dramatically decreased the accumulation of arsenic in HepG2 cells and inhibited the cellular As^{3+} methylation. DMPS did not selectively inhibit the second step methylation but promoted the excretion of cellular As^{3+} and MMA^{3+}. To evaluate the mechanism for the inhibitory effects of DMPS on the biomethylation of As^{3+}, we investigated the expression of human arsenic (III) methyltransferase (hAS3MT) in HepG2 cells, the accumulation of cellular ROS, and the *in vitro* enzymatic methylation of As^{3+} and MMA^{3+}. The results suggested that DMPS inhibited the biomethylation through down-regulating the expression of hAS3MT and competitive coordinating with As^{3+} and MMA^{3+}.

2. Materials and methods

Caution: Handing arsenicals requires strict safeguards for the potential risk of arsenic compounds [7].

2.1. Reagents and cell culture

Anti-hAS3MT rabbit mAb (Sigma) and anti-β-Actin mouse mAb (Beyotime) were used in the experiments. BSA, DMPS, and $NaAsO_2$ were purchased from Sigma. Disodium methylarsonate and monomethylarsonic acid (MMA^{5+}) were bought from J&K Chemical Ltd. HepG2 cells were kindly provided by Dr. X. D. Han (Medical School, Nanjing University, China). HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml blasticodin (KeyGEN Biotech, China) at 37 °C under a 5% CO_2 atmosphere.

2.2. Preparation of MMA^{3+} and recombinant hAS3MT

MMA^{3+} was prepared by reducing MMA^{5+} with 10 molar equivalents of cysteine in deaerated ddH_2O at 95 °C for 60 min. The purity of MMA^{3+} was confirmed by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) as previously research [23].

The modified hAS3MT gene was cloned into BamHI-SalI restriction sites of pET-32a vector (Novagen). The recombinant plasmids of pET-32a-*hAS3MT* was transformed into *Escherichia coli* BL2(DE3) pLysS and then expressed at 25 °C. Details of the purification, confirmation of hAS3MT were shown in previous works [24,25].

2.3. Analysis of arsenic species

HepG2 cells were plated into 60 mm culture dishes (2×10^6 cells/dish) and cultured for 12 h. The cells were then treated with different concentrations of As^{3+} (1, 5, 10 and 25 μM), or co-treated with DMPS (0.02, 0.2, 1.0 or 2.0 mM) and 25 μM As^{3+}. Following 24 h of incubation, the cells were washed twice with PBS, digested by trypsin, and counted. The counted cells were harvested in 1.0 ml mixture containing 0.4 ml H_2O_2 and 0.6 ml Tris-HNO_3 (50 mM, pH7.4) and digested at 75 °C for 2 h. Digested samples were filtered through a 0.22 μm pore membrane and diluted with deionized water for analysis. Total arsenic concentration was determined by ICP-MS (Elan 9000) [24,26].

HPLC (PRP X-100, Hamilton) was used to separate arsenic species in culture medium and HepG2 cells. Arsenic metabolites in 1.0 ml culture medium were collected and then treated with 0.1 mM H_2O_2 at 75 °C for 2 h. The concentrations of each species were calculated according the standard curves [23,25,27].

2.4. Determination the cellular ROS by 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA)

The experiments were performed on BD LSRL Fortessa flow cytometer according to the manufacturer's protocol of ROS assay kit (Beyotime) [28]. In brief, after treatment with As^{3+} (25 μM), DMPS (0.02 mM or 0.2 mM), or a combination of As^{3+} and DMPS for 24 h, HepG2 cells were collected, washed with PBS, and incubated in DMEM medium containing 10 μM DCFH-DA at 37 °C for 30 min. Then, the cells were washed twice to remove excrescent probes. Obtained data were analyzed by BD FACSDiva software.

2.5. Cell viability assay

WST-1 cell proliferation assay kit (KeyGEN Biotech, China) was used to measure the viability of HepG2 cells. HepG2 cells were seeded at a density of 2×10^4 cells/ml in a 96 well culture plate for 12 h before drug treatment. The seeded cells were exposed on various concentrations of As^{3+} and DMPS either alone or in combination for 24 h. Untreated cells served as control. After drug treatment, the cells were washed twice with phosphate buffer (PBS) and cultured in fresh medium. Cell proliferation was measured by WST-1 according to the manufacturer's protocol.

2.6. Flow cytometry

Cells were treated with DMPS (0.02 or 0.2 mM), 25 μM As^{3+}, or a combination of DMPS and As^{3+}. Apoptosis was measured after 24 h using the Annexin V-FITC and PI apoptosis detection kit (KeyGEN Biotech, China). HepG2 cells were digested from the dishes and washed twice by Ca^{2+}-free PBS. The washed cells were stained with Annexin V-FITC for 5 min and then were dyed with propidium iodide (PI) for 15 min. The double stained cells were analyzed by flow cytometer and the data were analyzed by BD FACSDiva software.

2.7. RT-PCR analysis

Total RNA was isolated from HepG2 cells by RNAiso Plus (Takara-Bio). The concentration and purity of isolated total RNA was determined on a trace nucleic acid protein measurement instrument (NanoDrop ND-1000). To prepare cDNA, 2 μg of total RNA was used to perform the reverse transcription with the PrimeScript RT reagent (Takara-Bio) according to the manufacturer's instructions. The transcribed cDNA (2 μL) was used to PCR amplification with specific primers of HMOX1, hAS3MT, and β-Actin genes. PCR was started with the initial denaturation at 94 °C for 5 min. Thirty cycles were carried out using the following conditions: 30 s denaturation at 94 °C, 30 s annealing at 52 °C (β-Actin), 57 °C (HMOX1), or 51 °C (hAS3MT), and 30 s extension at 72 °C. The sizes of the products of HMOX1, hAS3MT, and β-Actin were 181, 214 and 430 bp, respectively. The PCR products were separated through electrophoresis on a 1% agarose gel stained with 2 μg/ml ethidium bromide. The

separated bands were imaged on a GelDoc XR System (Bio-Rad). Primer sequences were as follows: HMOX1; sense: CTTTG-AGGAGTTGCAGGAGC, antisense: TGTAAGGACCCATCGGAGAA. hAS3MT; sense: ATGGCTTCCAGGCATCTAA, antisense: GGCAG-TTCAAGGCTCGTAT. β-Actin; sense: GACCTGACTGACTACCTC, antisense: TCTTCATTGTGCTGGGTGC.

2.8. Western-blot analysis

After 24 h treatment with As^{3+}, DMPS, or a combination of As^{3+} and DMPS, the total cellular protein were extracted in ice-cold lysis buffer (Beyotime) with 1.0 mM PMSF. The protein was collected through centrifugation and quantified according to BCA protein quantification kit (Beyotime). 30 μg of total protein of each sample was separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel and then transferred onto a PVDF membrane (Millipore). Subsequent to blocking with 5% skim milk in PBS-Tween overnight, membranes were successively incubated with the primary and secondary antibodies. Bound antigen on the PVDF membrane was visualized using the chemiluminescent HRP substrate (Millipore Corporation Billerica, USA). All the experiments were repeated three or more times.

2.9. Effects of DMPS on the recombinant hAS3MT catalyzed arsenic methylation

As^{3+} reaction system and MMA^{3+} reaction system were used in the experiments. As^{3+} reaction system contains 2.0 μM recombinant hAS3MT, 2.0 μM As^{3+}, 7.0 mM GSH and 0.5 mM S-Adenosyl-L-methionine (AdoMet). MMA^{3+} reaction system only differs in substrate: 2.0 μM MMA^{3+}. The effects of DMPS on the enzymatic methylation of As^{3+}/MMA^{3+} were determined by incubating different concentrations of DMPS (2–100 μM) with As^{3+} or MMA^{3+} reaction system at 37 °C for 90 min. Meanwhile, we designed velocity assay to clarify the inhibitory effects of DMPS. For the As^{3+} methylation, DMPS (2, 4, 6, and 8 μM) at different concentrations were added to As^{3+} (0, 2, 4, 6, and 8 μM) at fixed concentrations before incubation at 37 °C for 30 min. For the MMA^{3+} methylation, different concentrations of DMPS were added to fixed concentrations of MMA^{3+} (0, 2, 4, 6, and 8 μM) before incubation at 37 °C for 30 min. The reactions were stopped by H_2O_2 and analyzed by HPLC-ICP-MS [24,25,27]. The methylation rates were calculated as mole equivalents of methyl groups transferred from AdoMet to trivalent arsenic [25,27].

2.10. Statistical analysis

SPSS 19.0 was used to analyze the data by Student's *t*-test. A value of $P < 0.05$ was considered statistical significance.

3. Results

3.1. DMPS inhibits arsenic uptake

Effects of DMPS on arsenic uptake were determined by ICP-MS [26]. As shown in Fig. 1A, the contents of arsenic in HepG2 cells were increased with the increasing of As^{3+} concentration (1.0–25 μM). DMPS inhibited As^{3+} getting into the cells. When the cells were co-treated with As^{3+} (25 μM) and DMPS (0.02, 0.2, 1.0, or 2.0 mM), cellular arsenic was decreased with the increasing of DMPS concentration from 0.02 mM to 2.0 mM (Fig. 1A). Further analysis of the contents of cellular arsenic in total arsenic suggested that DMPS at high concentration (>1.0 mM) dramatically decreased the accumulation of arsenic in HepG2 cells. The cellular arsenic was decreased from 5.72% to 0.13% when the cells were treated with 2.0 mM DMPS (Fig. 1B).

3.2. DMPS inhibits the cellular As^{3+} methylation

To clarify the effects of DMPS on the biotransformation of arsenic, we analyzed the metabolites of arsenic in HepG2 cells and culture medium by HPLC-ICP-MS [24,25]. Because trivalent arsenicals are readily bound to cysteine residues of proteins, samples were treated with H_2O_2 to release trivalent arsenic species from proteins and convert them to pentavalent forms [24,25,27].

After 24 h treatment with 25 μM As^{3+}, HepG2 cells transferred As^{3+} to methylated arsenicals. The peak corresponding to dimethylarsinic acid (DMA^{5+}) in cell lysates were not detected after the metabolites were oxidized by H_2O_2 (Fig. 2A). We detected both monomethylarsonic acid (MMA^{5+}) and DMA^{5+} in culture medium (Fig. 2C). Subsequently, we investigated the effects of DMPS on the cellular As^{3+} methylation. DMPS (>0.2 mM) absolutely inhibited the biomethylation of As^{3+} in HepG2 cells. However, we detected the metabolites of As^{3+} after the cells were co-treated with 0.02 mM DMPS and 25 μM As^{3+} for 24 h (Fig. 2B and D). 0.02 mM DMPS obviously inhibited the cellular methylation of As^{3+}, where the generated MMAs were decreased from 16.65 ng to 9.72 ng, and DMAs were decreased from 6.32 ng to 2.65 ng (Table 1). Meanwhile, DMPS (0.02 mM) promoted inorganic arsenic and MMAs out of the

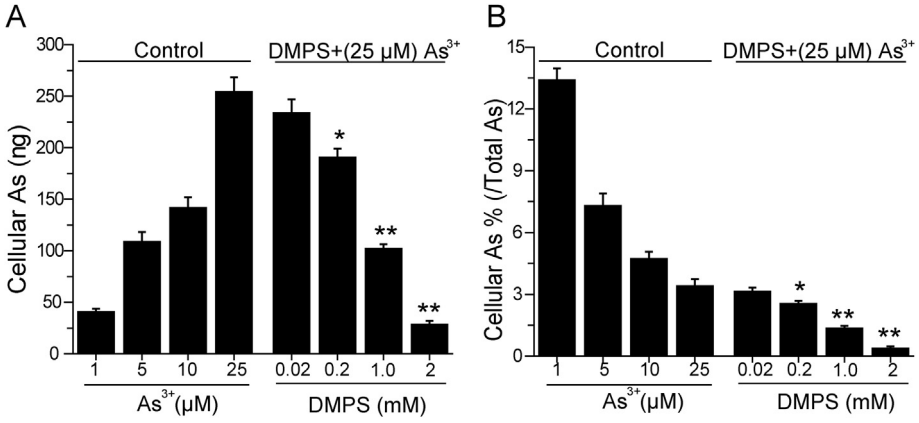

Fig. 1. Effects of DMPS on arsenic uptake. (A) Cellular total arsenic determined by ICP-MS. (B) The contents of cellular arsenic after As^{3+} and DMPS treatments. DMPS + As^{3+} represents the cells were co-treated with As^{3+} (25 μM) and DMPS (0.02, 0.2, 1.0 or 2.0 mM). Error bars represent S.D. from the mean of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to 25 μM As^{3+} treated cells.

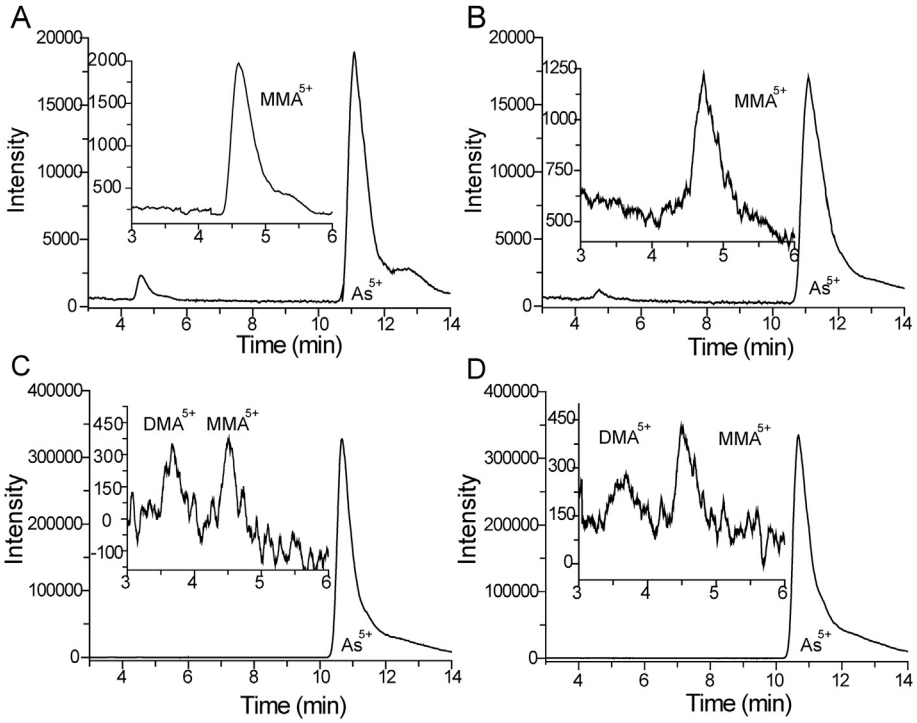

Fig. 2. Elution profiles of arsenic species determined by HPIC-ICP-MS. (A) Arsenic metabolites in 25 μM As^{3+} treated HepG2 cells. (B) The metabolites in culture medium after As^{3+} treatment. (C) Arsenic metabolites in 25 μM As^{3+} and 0.02 mM DMPS co-treated cells. (D) The metabolites in culture medium after As^{3+} and DMPS co-treatment. After drugs treatment, the arsenic metabolites in cells and culture medium were oxidized by H_2O_2 to their pentavalent forms. The figure shows a representative experiment of three independent experiments.

cells, where the cellular inorganic arsenic was decreased from 231.43 ng to 169.53 ng and the cellular MMAs were decreased from 11.25 ng to 4.88 ng (Table 1). DMPS inhibited As^{3+} entering HepG2 cells for further methylation and promoted arsenic metabolites out of the cells.

3.3. DMPS down-regulates the expression of hAS3MT induced by arsenic

To determine the reasons for the inhibition of DMPS in cellular As^{3+} methylation, we analyzed the expression of hAS3MT in HepG2 cells by RT-PCR and Western-blots. DMPS did not modulate the mRNA of hAS3MT (Fig. 3A). The up-regulation of As^{3+} on the expression of hAS3MT in gene level was not obvious (Fig. 3A). However, As^{3+} significantly up-regulated the expression of hAS3MT in protein level (Fig. 3B). The results of Western-blots suggested that DMPS strongly inhibited the expression of hAS3MT induced by As^{3+} (Fig. 3B).

Table 1
DMPS inhibits As^{3+} entering HepG2 cells for the enzymatic methylation.

HepG2 cells H_2O_2(+)	As^{3+} (25 μM)		(0.02 mM) DMPS+ (25 μM) iAs^{3+}	
	Cell lysate	Medium	Cell lysate	Medium
As^{5+} (ng)	231.43 ± 8.63	6773.2 ± 58.3	169.53 ± 12.11	6922.0 ± 43.9
MMA^{5+} (ng)	11.25 ± 1.62	5.40 ± 0.38	4.88 ± 0.46	4.84 ± 0.32
DMA^{5+} (ng)	ND	6.32 ± 0.42	ND	2.65 ± 0.38
Total As (ng)	242.68	6784.92	174.41	6929.49
As recovery (%)	94.33%		95.36%	

The yields of arsenicals in Fig. 3 were calculated according to standard curves using Origin 8.0. ND represents the species were not detected.

3.4. DMPS eliminates cellular ROS

To further investigate the mechanism for the inhibitory effects of DMPS on the cellular biotransformation of As^{3+}, we detected the cellular ROS by flow cytometer. DMPS decreased the level of cellular ROS. The 2′,7′-dichlorofluorescin (DCF) positive cell rates were decreased with the increasing of DMPS concentration from 0.02 mM to 0.2 mM (Fig. 4A). Meanwhile, DMPS eliminated As^{3+} generated ROS in HepG2 cells (Fig. 4B). Previous research showed that As^{3+} significantly up-regulated the expression of ROS-related gene HMOX1 [29]. Therefore, we analyzed the expression of HMOX1 to reflect the effects of DMPS on As^{3+}-generated ROS (Fig. 4C). DMPS down-regulated the expression of HMOX1, and inhibited the expression of HMOX1 induced by As^{3+} (Fig. 4D).

3.5. DMPS inhibits the ROS-related changes in HepG2 cells

As^{3+}-induced accumulation of ROS could cause cell apoptosis. To gain insight into the effects of DMPS on As^{3+}-induced apoptosis in HepG2 cells, we firstly investigated the viability of HepG2 cells using WST-1 assay. After 24 h treatment, 25 μM As^{3+} decreased the viability of HepG2 cells to 74.6% (Fig. 5). DMPS (0.02 and 0.2 mM) did not inhibit the growth of HepG2 cells but slightly increased. Further analysis suggested that DMPS could recover As^{3+}-induced growth inhibition. DMPS increased the proliferation of As^{3+}-treated cells from 74.6% to 81.0% (0.02 mM DMPS) or 85.4% (0.2 mM DMPS) (Fig. 5).

Subsequently, we studied the apoptosis of HepG2 cells by flow cytometry. As shown in Fig. 6, DMPS prevented the cells from apoptosis. The contents of apoptotic cells were decreased to 8.5% in 0.02 mM DMPS-treated group and 4.8% in 0.2 mM DMPS-treated group. Meanwhile, DMPS inhibited As^{3+}-induced apoptosis in

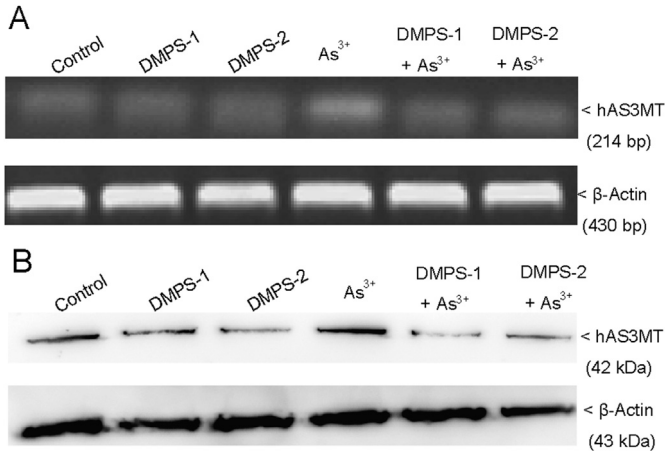

Fig. 3. Effects of DMPS on the expression of hAS3MT in HepG2 cells. (A) The expression of hAS3MT analyzed by RT-PCR. (B) The expression of hAS3MT analyzed by Western blots. The figure shows a representative experiment of at least three independent experiments.

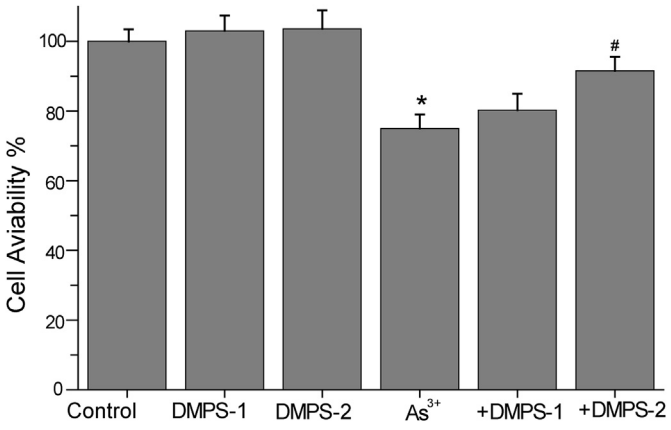

Fig. 5. Effects of DMPS and As^{3+} on the viability of HepG2 cells. +DMPS-1 and +DPMS-2 respectively represent the cells were co-treated with 0.02 mM DMPS and 25 μM As^{3+}, and 0.2 mM DMPS and 25 μM As^{3+} for 24 h. Error bars represent S.D. from the mean of three independent experiments. $^{*}P < 0.05$ compared to control. $^{\#}P < 0.05$ compared to 25 μM As^{3+} treated cells.

HepG2 cells. Compared with As^{3+}-treated cells, 0.02 mM DMPS decreased the apoptotic rates from 34.7% to 27.6%, and 0.2 mM DMPS decreased the contents from 34.7% to 15.7% (Fig. 6).

3.6. *DMPS competitively inhibits the enzymatic methylation of As^{3+} and MMA^{3+}*

DMPS down-regulated the expression of hAS3MT induced by As^{3+}, but did not increase the cellular ROS to oxide the trivalent arsenicals and inactivate hAS3MT. Herein, we constructed recombinant hAS3MT to study the effects of DMPS on the methylation of As^{3+} and MMA^{3+} *in vitro*. DMPS dramatically inhibited the As^{3+} methylation at low concentration. The enzymatic methylation of As^{3+} was absolutely blocked by 40 μM DMPS. The IC_{50} value of DMPS for the As^{3+} methylation was 6.0 μM (Fig. 7A). Compared with the As^{3+} methylation, the inhibitory effect of DMPS on the second step methylation of MMA^{3+} was weaker. The IC_{50} values of

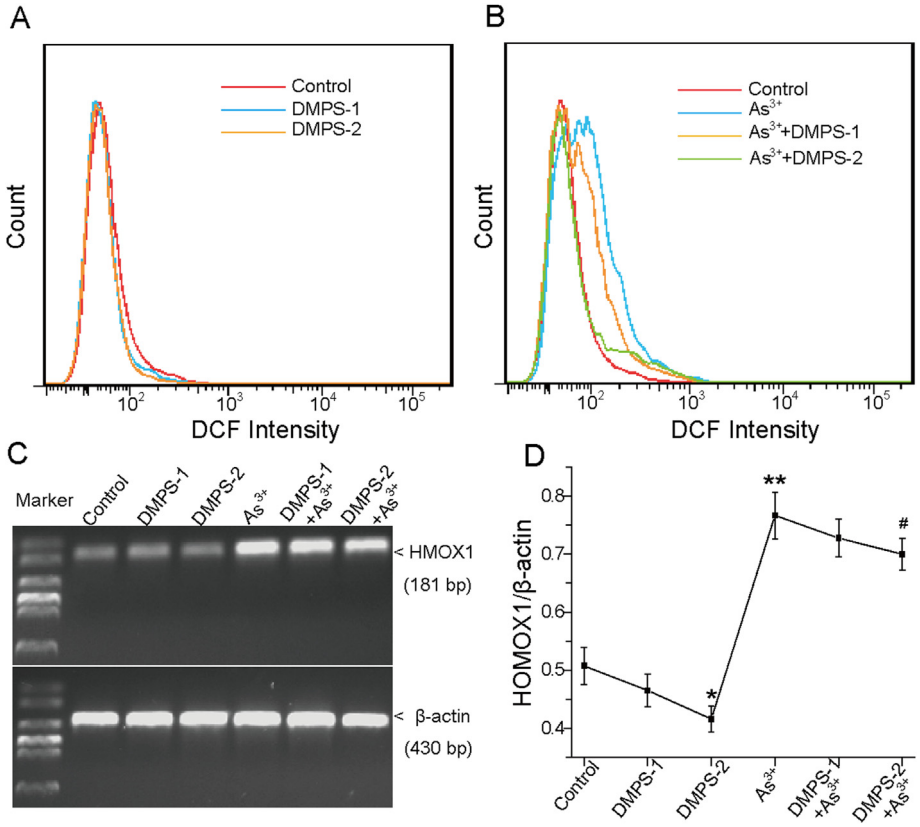

Fig. 4. Accumulation of ROS in HepG2 cells. (A) Effects of DMPS on cellular ROS analyzed by flow cytometry. (B) Effects of DMPS on As^{3+} generated ROS in HepG2 cells. (C) The expression of HMOX1 analyzed by RT-PCR. (D) Percentage of relative intensity obtained the corresponding RT-PCR. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to control. $^{\#}P < 0.05$ compared to 25 μM As^{3+} treated group. Error bars represent S.D. from the mean of three independent experiments.

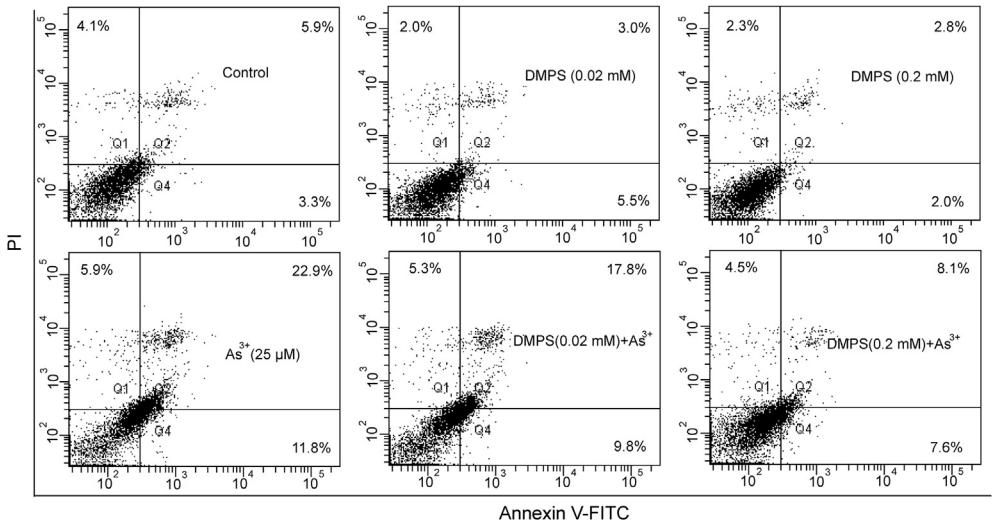

Fig. 6. Effects of DMPS on As^{3+} induced apoptosis in HepG2 cells measured by Annexin V–FITC and PI double staining. HepG2 cells were treated with DMPS (0.02 mM or 0.2 mM), As^{3+} (25 μM), or a combination of DMPS and As^{3+}. Q_1 and Q_3 respectively represent the contents of death cells and living cells. Q_2 and Q_4 were used to calculate apoptotic cells. The figure shows a representative experiment of three independent experiments.

DMPS for the MMA^{3+} methylation was 15 μM (Fig. 7B). DMPS did not selectively inhibit the second step methylation from MMA^{3+} to DMA^{3+}.

Competitive, uncompetitive, noncompetitive, and mixed are the four possible mechanisms proposed to describe the types of inhibition [30,31]. The function derived from the steady-state model was used to analyze the inhibition of DMPS with respect to As^{3+} or MMA^{3+} in the enzymatic methylation [30]:

$$V_0 = V_{max}[S]/((1 + [I]/K_I)\cdot K_M + (1 + [I]/K_{IS})\cdot[S]) \quad (1)$$

K_M, K_I, K_{IS}, S, I, and V_0 respectively represent for Michaelis constant, dissociation constant of enzyme–inhibitor complex, dissociation constant of enzyme-substrate-inhibitor complex, substrate, inhibitor, and initial velocity. As shown in double–reciprocal graphs, the lines for 1/v against $1/As^{3+}$ crossed at y-axis, and the lines for v versus As^{3+} were well fitted by Eq. (1) (Fig. 8A and B). The fitted value of K_I was 2.6 μM and K_{IS} was infinity, which suggested that DMPS competitively inhibited As^{3+} involving in the enzymatic methylation. Subsequently, we studied the mechanism for the inhibition of DMPS in MMA^{3+} methylation. The plots for 1/v against $1/MMA^{3+}$ mixed at vertical, and lines for v against MMA^{3+} were well fitted by the function of competitive inhibition (Fig. 8C and D). K_I of DMPS with respect to MMA^{3+} was 3.99 μM and K_{IS} was infinity. The kinetic results suggested that DMPS competitively inhibited the recombinant hAS3MT catalyzed methylation of As^{3+} and MMA^{3+} [30,31]. The inhibitory effect of DMPS on the As^{3+} methylation was more obviously than on the second step methylation of MMA^{3+} [30].

4. Discussion

DMPS serves as antidote for arsenic poisoning [18,19]. Researches on the antitoxic mechanisms of DMPS regarded that DMPS modulated the biomethylation of arsenic and excreted MMA^{3+} in the form of DMPS-MMA^{3+} [20–22]. However, the mechanism was proposed through analyzing the excretion of arsenic species. The effects of DMPS on arsenic poisoning should be related to its functions in the biotransformation of arsenic. Herein, we selected HepG2 cells as model to study the effects of DMPS on the biomethylation of As^{3+}. Meanwhile, we constructed recombinant hAS3MT to investigate the mechanism for the inhibition of DMPS.

DMPS showed low biological toxicity and promoted the excretion of arsenic [18,19]. In this work, we found that DMPS at low concentration could increase the viability of HepG2 cells and prevent arsenic uptake. Moreover, DMPS dramatically inhibited the

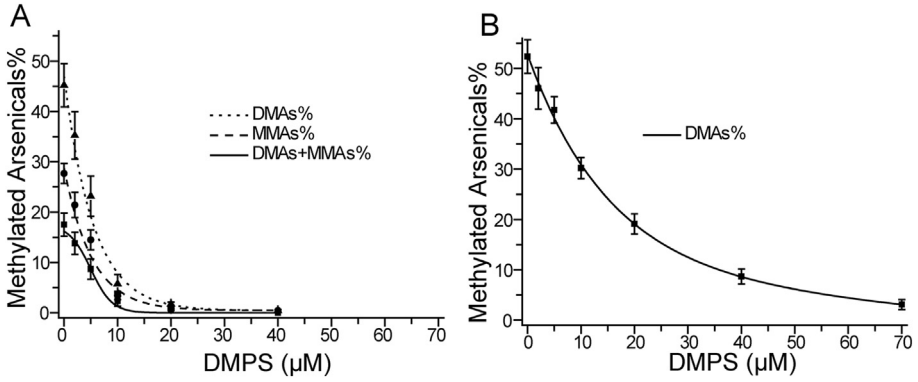

Fig. 7. Effects of DMPS on recombinant hAS3MT catalyzed arsenic methylation *in vitro*. The effects of DMPS on the enzymatic methylation of As^{3+} (A) and MMA^{3+} (B). Error bars represent S.D. from the mean of three independent experiments.

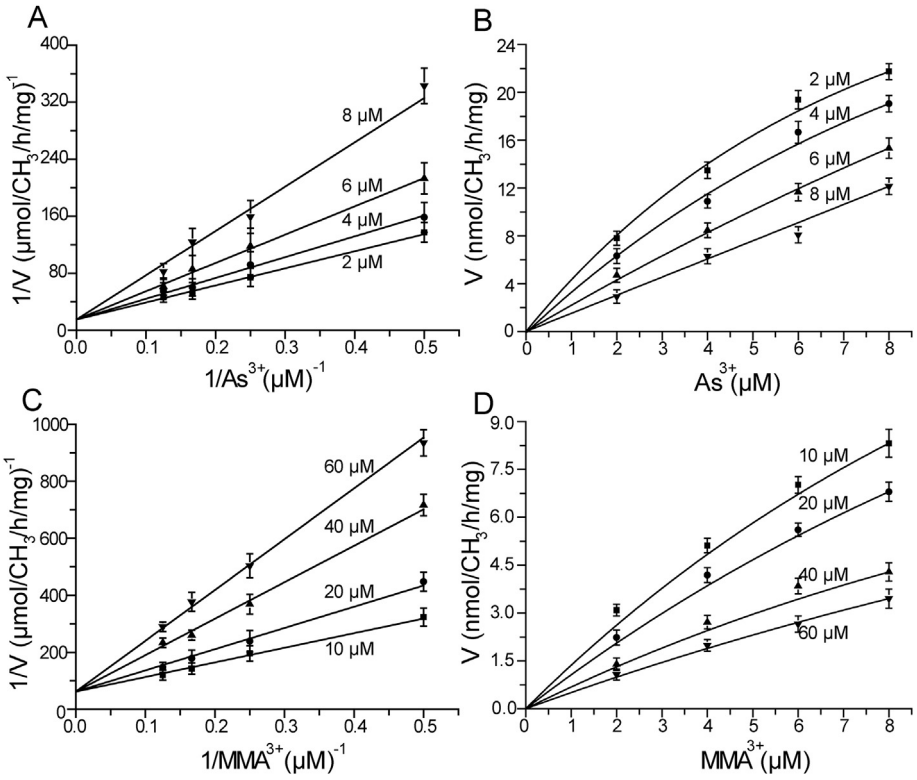

Fig. 8. Kinetic analysis of the mechanism for the inhibition of DMPS in the enzymatic methylation. (A) Double reciprocal plots and (B) *v* against As^{3+} plots for the inhibition of DMPS in As^{3+} methylation. (C) Double reciprocal plots and (D) *v* against [MMA^{3+}] plots for the inhibition of DMPS in MMA^{3+} methylation. Error bars represent S.D. from the mean of three independent experiments.

cellular conversion of arsenic from As^{3+} to MMA^{3+} and promoted MMA^{3+} getting out of the cells. Similar to the results in clinical trials [20–22], DMPS prevented As^{3+} from getting into HepG2 cells, inhibited the cellular arsenic methylation, and promoted the metabolites getting out of the cells.

Studies *in vitro* suggested that the enzymatic methylation of arsenic was a reductant-related reaction [14,25]. The expression of hAS3MT and the levels of cellular GSH play key roles in the cellular biomethylation of arsenic. To clarify the inhibitory effects of DMPS on the cellular As^{3+} methylation, we considered three aspects. First, DMPS may down-regulate the expression of hAS3MT in HepG2 cells to inhibit the enzymatic methylation. The results proved that DMPS inhibited the expression of hAS3MT induced by As^{3+}. Second, DMPS may induce the accumulation of ROS [32]. The generated ROS could eliminate the cellular GSH, oxidize the trivalent arsenicals, and inactive hAS3MT, which would block the enzymatic methylation of As^{3+}. However, DMPS decreased the cellular ROS and eliminated the ROS induced by As^{3+}. Third, we regarded that DMPS might directly inhibit the hAS3MT catalyzed As^{3+} methylation. The *in vitro* experiments showed that DMPS inhibited the hAS3MT catalyzed As^{3+} methylation at very low concentration but did not selectively inhibit the second step methylation from MMA^{3+} to DMA^{3+}. DMPS competitively inhibited As^{3+} and MMA^{3+} involving in the enzymatic methylation. There are two explanations for the competitive inhibition: inhibitor competitively blocks the binding sites for substrate, and inhibitor competitively coordinates with substrate to prevent substrate binding to the enzyme. Cysteine residues of hAS3MT are the active sites of arsenic [23–25,33,34]. Thus, we regarded that DMPS competitively coordinated with As^{3+} and MMA^{3+} to prevent them binding to the active site cysteine residues of hAS3MT.

Above all, DMPS competitively coordinated with trivalent arsenicals, which could inhibit As^{3+} up-regulating the expression of hAS3MT and prevent As^{3+} and MMA^{3+} involving in the enzymatic methylation. The enzymatic methylation of arsenic itself and the methylated arsenicals could generate ROS and induce oxidative damages [16,17,35]. We found that DMPS eliminated the cellular ROS and inhibited ROS-related changes in HepG2 cells. Thus, we proposed that DMPS could protect human body from As^{3+}-induced oxidative.

5. Conclusions

In summary, we studied the effects of DMPS on the biotransformation of arsenic in HepG2 cells. The results confirmed that DMPS prevented As^{3+} from getting into cells, and promoted arsenic metabolites getting out of cells. DMPS inhibited the biomethylation of As^{3+} through down-regulating the expression of hAS3MT induced by As^{3+} and competitive coordinating with trivalent arsenicals. Beyond inhibiting the uptake and metabolism of arsenic, DMPS could eliminate arsenic-induced accumulation of ROS, which might contribute to the antidotal effects of DMPS on arsenic posing.

Author agreement

Material submitted is original, all authors agreed to publish the article.

Conflict of interest

The authors declared that there is no conflict of interest.

Acknowledgments

This research was supported by the National Basic Research Program of China (2013CB922102) and the National Natural Science Foundation of China (21275072 and 21201101).

References

[1] X. Cui, Y. Kobayashi, M. Akashi, R. Okayasu, Metabolism and the paradoxical effects of arsenic: carcinogenesis and anticancer, Curr. Med. Chem. 15 (2008) 2293–2304.
[2] D. Douer, M.S. Tallman, Arsenic trioxide: new clinical experience with an old medication in hematologic malignancies, J. Clin. Oncol. 23 (2005) 2396–2410.
[3] A.M. Florea, D. Büsselberg, The two opposite facets of arsenic: toxic and anticancer drug, J. Local Glob. Health Sci. 66 (2013) 1.
[4] Z.Y. Wang, Arsenic compounds as anticancer agents, Cancer Chemother. Pharmcol. 48 (suppl 1) (2001) S72–S76.
[5] X.W. Zhang, X.J. Yan, Z.R. Zhou, F.F. Yang, Z.Y. Wu, H.B. Sun, W.X. Liang, A.X. Song, V. Lallemand-Breitenbach, M. Jeanne, Q.Y. Zhang, H.Y. Yang, Q.H. Huang, G.B. Zhou, J.H. Tong, Y. Zhang, J.H. Wu, H.Y. Hu, H. de Thé, S.J. Chen, Z. Chen, Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML, Science 328 (2010) 240–243.
[6] R.N. Ratnaike, Acute and chronic arsenic toxicity, Postgrad. Med. J. 79 (2003) 391–396.
[7] S. Tapio, B. Grosche, Arsenic in the aetiology of cancer, Mutat. Res. 612 (2006) 215–246.
[8] J.C. Saha, A.K. Dikshit, M. Bandyopadhyay, K.C. Saha, A review of arsenic poisoning and its effects on human health, Crit. Rev. Env. Sci. Tec. 29 (1999) 281–313.
[9] D.J. Thomas, M. Styblo, S. Lin, The cellular metabolism and systemic toxicity of arsenic, Toxicol. Appl. Pharmcol. 176 (2001) 127–144.
[10] D.J. Thomas, S.B. Waters, M. Styblo, Elucidating the pathway for arsenic methylation, Toxicol. Appl. Pharmcol. 198 (2004) 319–326.
[11] D.J. Thomas, Molecular processes in cellular arsenic metabolism, Toxicol. Appl. Pharmcol. 222 (2007) 365–373.
[12] D.J. Thomas, G.M. Nava, S.Y. Cai, J.L. Boyer, A. Hernández-Zavala, H.R. Gaskins, Arsenic (+ 3 oxidation state) methyltransferase and the methylation of arsenicals in the invertebrate chordate ciona intestinalis, Toxicol. Sci. 113 (2010) 70–76.
[13] W.R. Cullen, B.C. McBride, J. Reglinski, The reaction of methylarsenicals with thiols: some biological implications, J. Inorg. Biochem 21 (1984) 179–193.
[14] W.R. Cullen, Chemical mechanism of arsenic biomethylation, Chem. Res. Toxicol. 176 (2014) 127–144.
[15] T. Hayakawa, Y. Kobayashi, X. Cui, S. Hirano, A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19, Arch. Toxicol. 79 (2005) 183–191.
[16] S.J. Flora, G. Flora, G. Saxena, M. Mishra, Arsenic and lead induced free radical generation and their reversibility following chelation, Cell. Mol. Biol. 53 (2007) 26–47.
[17] L. Lin, T.M. Stringfield, X. Shi, Y. Chen, Arsenite induces a cell stress-response gene, RTP801, through reactive oxygen species and transcription factors Elk-1 and CCAAT/enhancer-binding protein, Biochem. J. 392 (2005) 93–102.
[18] H.V. Aposhian, DMSA and DMPS–water soluble antidotes for heavy metal poisoning, Annu. Rev. Pharmacol. 23 (1983) 193–215.
[19] R. Heinrich-Ramm, H. Schaller, J. Horn, J. Angerer, Arsenic species excretion after dimercaptopropanesulfonic acid (DMPS) treatment of an acute arsenic trioxide poisoning, Arch. Toxicol. 77 (2003) 63–68.
[20] H.V. Aposhian, A. Arroyo, M.E. Cebrian, L.M. del Razo, K.M. Hurlbut, R.C. Dart, D. Gonzalez-Ramirez, H. Kreppel, H. Speisky, A. Smith, M.E. Gonsebatt, P. Ostrosky-Wegman, M.M. Aposhian, DMPS-arsenic challenge test. I: increased urinary excretion of monomethylarsonic acid in humans given dimercaptopropane sulfonate, J. Pharmacol. Exp. Ther. 282 (1997) 192–200.
[21] H.V. Aposhian, B. Zheng, M.M. Aposhian, X.C. Le, M.E. Cebrian, W. Cullen, R.A. Zakharyan, M. Ma, R.C. Dart, Z. Cheng, P. Andrewes, L. Yip, G.F. O'Malley, R.M. Maiorino, W. Van Voorhies, S.M. Healy, A. Titcomb, DMPS-arsenic challenge test. II. Modulation of arsenic species, including monomethylarsonous acid (MMA(III)), excreted in human urine, Toxicol. Appl. Pharmcol. 165 (2000) 74–83.
[22] Z. Gong, G. Jiang, W.R. Cullen, H.V. Aposhian, X.C. Le, Determination of arsenic metabolic complex excreted in human urine after administration of sodium 2,3-dimercapto-1-propane sulfonate, Chem. Res. Toxicol. 15 (2002) 1318–1323.
[23] X.L. Li, Z.R. Geng, J.Y. Chang, S.P. Wang, X.L. Song, X. Hu, Z.L. Wang, Identification of the third binding site of arsenic in human arsenic(III) methyltransferase, PLoS One 8 (2013) e84231.
[24] Z.R. Geng, X.L. Song, Z. Xing, J.L. Geng, S.C. Zhang, X.R. Zhang, Z.L. Wang, Effects of selenium on the structure and function of recombinant human S-adenosyl-L-methionine dependent arsenic (+3 oxidation state) methyltransferase in E. coli, J. Biol. Inorg. Chem. 14 (2009) 485–496.
[25] S.P. Wang, X.L. Li, X.L. Song, Z.R. Geng, X. Hu, Z.L. Wang, Rapid equilibrium kinetic analysis of arsenite methylation catalyzed by recombinant human arsenic (+3 oxidation state) methyltransferase (hAS3MT), J. Biol. Chem. 287 (2012) 38790–38799.
[26] T. Watanabe, Y. Ohta, A. Mizumura, Y. Kobayashi, S. Hirano, Analysis of arsenic metabolites in HepG2 and AS3MT-transfected cells, Arch. Toxicol. 85 (2011) 577–588.
[27] X.L. Song, Z.R. Geng, X.L. Li, X. Hu, N.S. Bian, X.R. Zhang, Z.L. Wang, New insights into the mechanism of arsenite methylation with the recombinant human arsenic (+3) methyltransferase (hAS3MT), Biochimie 92 (2010) 1397–1406.
[28] E. Eruslanov, S. Kusmartsev, Identification of ROS using oxidized DCFDA and flow-cytometry, Methods Mol. Biol. 594 (2010) 57–72.
[29] D. Morse, A.M.K. Choi, Heme oxygenase-1: from bench to bedside, Am. J. Respir. Crit. Care Med. 172 (2005) 660–670.
[30] A. Cornish-Bowden, A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors, Biochem. J. 137 (1974) 143–144.
[31] Y. Wang, X. Liu, B. Schneider, E.A. Zverina, K. Russ, S.J. Wijeyesakere, C.A. Fierke, R.J. Richardson, M.A. Philbert, Mixed inhibition of adenosine deaminase activity by 1,3-dinitrobenzene: a model for understanding cell-selective neurotoxicity in chemically-induced energy deprivation syndromes in brain, Toxicol. Sci. 125 (2012) 509–521.
[32] K.Y. Jan, T.C. Wang, B. Ramanathan, J.R. Gurr, Dithiol compounds at low concentrations increase arsenite toxicity, Toxicol. Sci. 90 (2006) 432–439.
[33] X.L. Song, Z.R. Geng, J.S. Zhu, C.Y. Li, X. Hu, N.S. Bian, X.R. Zhang, Z.L. Wang, Structure-function roles of four cysteine residues in the human arsenic (+3 oxidation state) methyltransferase (hAS3MT) by site-directed mutagenesis, Chem. Biol. Interact. 179 (2009) 321–328.
[34] X.L. Song, Z.R. Geng, X.L. Li, Q. Zhao, X. Hu, X.R. Zhang, Z.L. Wang, Functional and structural evaluation of cysteine residues in the human arsenic (+3 oxidation state) methyltransferase (hAS3MT), Biochimie 93 (2011) 369–375.
[35] S.J. Flora, Arsenic-induced oxidative stress and its reversibility, Free Radic. Biol. Med. 51 (2011) 257–281.