Characterization of the Interaction of Galectin-1 with Sodium Arsenite

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · APRIL 2006

Impact Factor: 3.53 · DOI: 10.1021/tx0503348 · Source: PubMed

Characterization of the Interaction of Galectin-1 with Sodium Arsenite

Chen-Huan Lin,[†] Chi-Fei Huang,[†] Wen-Yih Chen,[‡] Yu-Ying Chang,[†] Wang-Hsien Ding,[§] Ming-Shen Lin,[‡] Szu-Hsun Wu,[§] and Rong-Nan Huang*,[†]

Department of Life Science, College of Science, National Central University & University System of Taiwan, Chung-Li, Taoyuan, Taiwan 32054, ROC, and Departments of Chemical & Materials Engineering and Chemistry, National Central University, Chung-Li, Taoyuan, Taiwan 32054, ROC

Received December 1, 2005

We previously showed that galectin-1 (GAL1) is an arsenic-binding protein. In the current study, we further characterize the interaction of GAL1 with sodium arsenite (As(III)). The GALl−As(III) complex was prepared from the cell extracts of GAL1-transfected *Escherichia coli* (*E. coli*) that were pretreated with As(III). The results of the circular dichroism (CD) spectrum of GAL1−As(III) exhibited a negative signal at around 205−210 nm, whereas that of GAL1 showed a negative signal at around 215−220 nm. This shift in the CD spectrum is indicative of a substantial change in the secondary structure arising from the binding of As(III) to the GAL1 protein. The UV absorptive spectrum of the GAL1−As(III) complex was significantly lower than that of GAL1 itself. A mobility shift binding assay showed that the GAL1−As(III) complex migrated closer than GAL1 toward the anode. Capillary electrophoretic analysis also showed that As(III) binding decreased the mobility of GAL1. These results further confirmed the structural change of the GAL1 complex with As(III). Furthermore, isothermal titration microcalometric studies showed that As(III) titration into the GAL1 protein solution was an endothermic process with absorption enthalpy (ΔH_{abs}) around 8−10 kJ/mol As(III). The affinity constant (K_d) of As(III) toward GAL1 was around 8.239 ± 2.627 μM as estimated by tryptophan (Trp) fluorescence quenching. However, the binding of As(III) did not significantly affect the biological activity of GAL1, since the GAL1−As(III) complex only partially lost its lectin activity. In addition, we show that GAL1-transfected KB cells accumulated more arsenic than did the parental cells. Taken together, these results suggest that GAL1 might serve as a target protein of As(III) in vivo, and the binding of GAL1 with As(III) could interfere with the excretion of As(III).

Introduction

Arsenite (As(III)) is a well-documented human carcinogen. Environmental exposure to arsenic from copper smelters and drinking water has been associated with a number of pathological problems, such as skin, bladder, and lung cancers (*1*, *2*). Arsenic exposure has also been related to the etiology of diabetes and cardiovascular disease (e.g., hypertension) (*3*−*6*). The exact biological targets of arsenic inside cells are not known. However, the binding of arsenic to tissue proteins has been proposed as the first step in the metabolism of arsenic (*7*). The adverse effects of arsenic on biological systems have also been proposed by its reaction with closely spaced cysteine residues on critical cellular proteins (*8*). Therefore, the binding of arsenic to proteins is an important determinant of arsenic toxicity (*9*).

Galectin-1 (GAL1) is a sugar-binding protein specific for β-galectosides. It exists as a soluble protein which forms a noncovalent homodimer and is expressed with a broad tissue distribution (*10*, *11*). GAL1 is found mainly in the cytosol of most cells in which it is synthesized (*12*) and then externalized to bind to β-galactosy-containing glycoconjugates on the cell surface and in the extracellular matrix (*13*−*15*). Since this lectin lacks an identifiable signal sequence, it does not appear to be secreted through normal secretory pathways (*16*, *17*).

Arsenic has been shown to exert at least some of its toxic effects through interactions with thiol groups of proteins, thereby modulating the activities of key regulatory proteins (*18*). GAL1 has six sulfhydryl groups which are not involved in the formation of the GAL1 dimer, but the free sulfhydryl groups are critical for its lectin-binding activity (*19*−*21*). GAL1, therefore, is an ideal model for probing the interaction of arsenic with proteins. We previously demonstrated that GAL1 is an arsenic-binding protein (*22*). In this study, GAL1 and GAL1−As(III) proteins were purified from GAL1-transfected *Escherichia coli* (*E. coli*) without or with As(III) treatment, and both proteins were used to probe the interactive mechanism of GAL1 with As(III).

Experimental Procedures

Cell Growth Assay and Arsenic Measurements. KB cells (oral epidermoid carcinoma cells) were obtained from American Type Culture Collection (Rockville, MD) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum and antibiotics as described previously (*23*). The cultures were maintained at 37 °C in a humidified gaseous phase of 95% air and 5% CO_2. Sodium arsenite ($NaAsO_2$; As(III)) was purchased from Sigma (Sigma, St. Louis, MO) and freshly prepared by dissolving it in double-distilled water. After being treated with As(III), KB cells were washed with PBS three times and subjected to survival assay or arsenic content determination

* Corresponding author. E-mail: lsrong@cc.ncu.edu.tw.
[†] Department of Life Science, National Central University & University System of Taiwan.
[‡] Department of Chemical & Materials Engineering, National Central University.
[§] Department of Chemistry, National Central University.

10.1021/tx0503348 CCC: $33.50
Published on Web 02/25/2006

using an atomic absorption spectrophotometer equipped with a hydride formation system (AAS, Perkin-Elmer AA100-FIAS-100, Norwalk, CT) according to the methods of Chang et al. (*22*).

GAL1 Transfection. The full-length GAL1 cDNAs were inserted into pQE vectors and pcDNA3.1 vectors to generate plasmids encoding GAL1 expressed in *E. coli* and KB cells, respectively. Details of the construction and expression of GAL1 were described previously (*22*).

Assay for Lectin Activity. Lectin-binding activity of the GAL1−As(III) complex was determined by a solid-phase binding assay according to Mazurek (*24*). Briefly, individual wells of a 96-well polystyrene microtiter plate (Nunc) were coated with Engelbreth−Holm−Swarm sarcoma laminin (10 μg/mL and 100 μL/well dissolved in phosphate-buffered saline (PBS)) at 4 °C overnight. The nonspecific protein-binding sites were saturated with 3% bovine serum albumin (BSA) made in PBS for 1 h at room temperature. GAL1, oxidized GAL1, or GAL1−As(III) samples in 0.5% BSA solution were then added to the coated wells and incubated for 1 h at 37 °C. The wells were next incubated with anti-GAL1 antibody in 0.5% blocking buffer for 1 h at 37 °C, followed by incubation with alkaline phosphatase-conjugated anti-mouse Ig (Kirkegarrd & Perry Laboratories) at room temperature for 1 h. At the end of the incubation, the protein-coated wells were developed with the *p*-nitrophenol phosphate (PNPP) substrate, and color development was monitored at 405 nm using a plate reader (Nunc, MRX). The wells were washed with PBST five times (10 min each) between each incubation step. Oxidized GAL1 was obtained by the air oxidation method with CuSO4 as described by Inagaki (*25*).

Heat Measurements. Measurement of the heat of the interaction of GAL1 with As(III) was essentially according to a previous description by Lin et al. (*26*) using VP-isothermal titration calorimetry (VP-ITC), which is a part of a Microcalorimeter (MicroCal). The MicroCal is a heat conduction type of microcalorimeter (MicroCal) controlled by computerized software called VP viewer2000. Heat leakage is simultaneously monitored in an isothermal system. Before the ITC measurement, the microreaction system was thermally equilibrated by insertion of a 1.8-mL reference cell which is filled with a GAL1 protein solution into the heat sink. Afterward, the As(III) solution was titrated into the GAL1 solution through an injector driven by a computer-controlled pump at a time interval of 40 min, and the output signal was recorded as power (*P*) versus time (*t*) and was integrated and quantified by the amount of adsorbed protein to give the enthalpy change of adsorption.

Circular Dichroism (CD) and UV Spectrometry. To clarify structural differences, the secondary structure of GAL1−As(III) was probed by CD and UV spectrometric measurements. In brief, GAL1 and GAL1−As(III) solutions (10 μg/mL dissolved in 20 mM PBS, pH 7.0) were scanned in a CD spectrometer (JASCO J-810) from 200 to 300 nm and a UV spectrometer (Beckman DU640) from 190 to 340 nm.

K_d Determination. The K_d of the interaction of As(III) with GAL1 was determined as described previously using tryptophan fluorescence (*27*, *28*) and capillary electrophoresis (*29*, *30*). The intrinsic tryptophan fluorescence of 0.25 μM GAL1 was examined at room temperature using a Hitachi F-4500 spectrofluorometer. The excitation wavelength typically was maintained at 295 nm, and emissions were monitored from 300 to 400 nm. Calculation of the binding constants (binding of As(III) to GAL1) was analyzed by curve fitting (KaleidaGraph) according to an equation described by Dunning Hotopp et al. (*28*).

The capillary electrophoresis was performed in a 60 cm × 50 μm fused-silica capillary mounted in a Beckman P/ACE MDQ CE system with UV detection at 208 nm. The capillary was sequentially preconditioned at 25 °C with methanol (10 min), 1 N HCl (10 min), deionized water (2 min), and 1 N NaOH (10 min), followed by rinsed with deionized water for 2 min and separation buffer (67 mM Na_2HPO_4, pH 7.4) for 10 min. Between runs, the capillary was conditioned with 0.1 N NaOH for 2 min and deionized water for 2 min. This procedure improved peak sharps and the reproducibility. The relative standard derivation of the migration time is less than 0.9%. The binding constant (binding of As(III) to GAL1) was calculated according to an equation described by Rundlett and Armstrong (*29*).

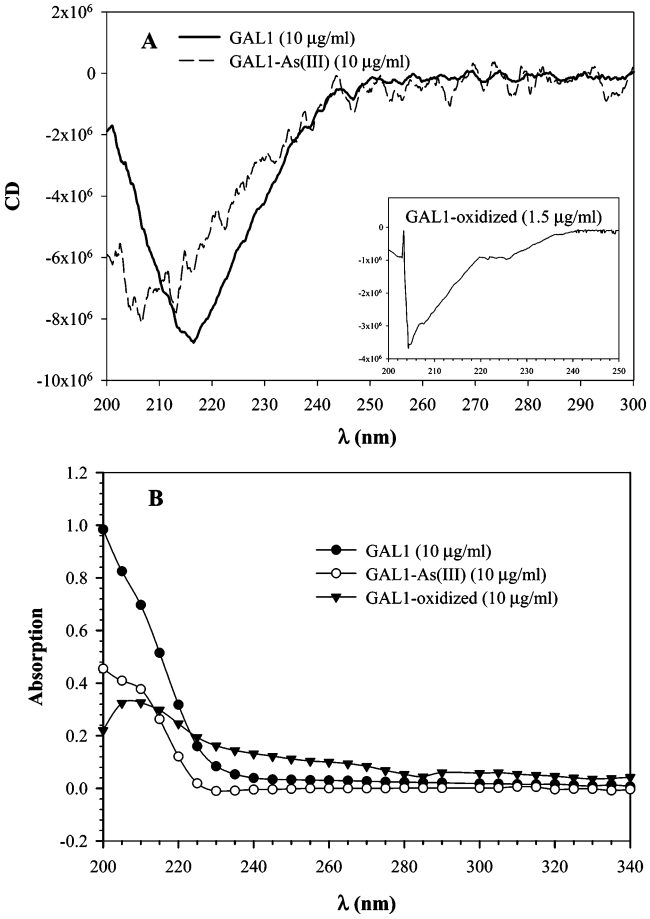

Figure 1. Effect of As(III) on the optical behavior of GAL1. GAL1, GAL1−As(III), and GAL1-oxidized proteins were dissolved in PBS and scanned with (A) circular dichroism (CD) and (B) UV spectrophotometers. Inset in panel A, the CD spectrum of GAL1-oxidized.

Gel-Mobility Shift Assay. Twenty-five micrograms each of GAL1 and GAL1−As(III) were respectively dissolved in 30 μL of PBS containing 20% glycerol and 1 mg/mL bromophenol blue. Samples were then electrophoresed in a 4% polyacrylamide gel (PAGE) at 50 V for 4 h with low ionic strength in Tris-base/acetate electrophoresis buffer (pH 8.5). At the end of electrophoresis, gels were stained with Coomassie brilliant blue R 250 for 12 h, followed by retention with ddH_2O for 12 h to visualize the protein bands.

Results

Effect of As(III) Binding on the Conformation of GAL1. The toxicity of As(III) has been proposed to result from its binding to proteins, and a number of thiol-containing proteins are altered by exposure to As(III). Our previous studies demonstrated that GAL1 is an As(III)-binding protein; we, therefore, further characterized whether the binding of GAL1 with As(III) alters the structure of GAL1. Differences in secondary structural features between GAL1 and the GAL1−As(III) complex were probed by measuring the CD spectrum from 170 to 240 nm. The CD spectrum of GAL−As(III) and oxidized GAL1 exhibited a negative signal at around 205−210 nm, whereas that of GAL1 showed a negative signal at around 215−220 nm (Figure 1A). This shift in the CD spectrum is indicative of a substantial change in the secondary structure resulting from the binding of As(III) to GAL1 proteins. We further scanned the UV absorption spectrum of the GAL1−

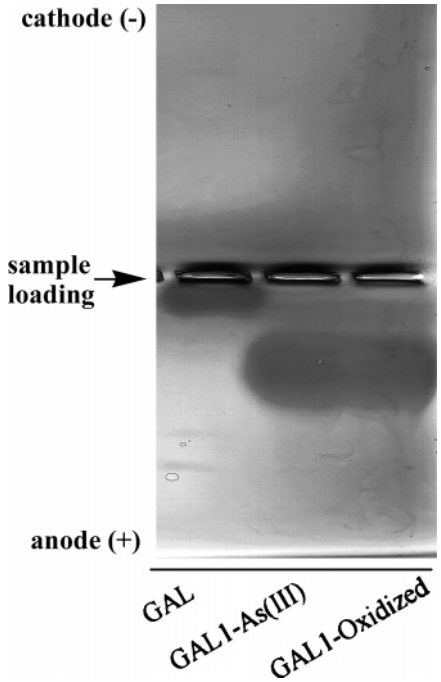

Figure 2. Effect of As(III) binding on the electrophoretic mobility of GAL1. GAL1, GAL1−As(III), and GAL1-oxidized were respectively dissolved in PBS containing glycerol and bromophenol blue and electrophoresed in 4% polyacrylamide gels with a low-ionic-strength as described in Experimental Procedures. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue R 250 for sample visualization.

As(III) complex, and the peak area of the GAL1−As(III) complex and oxidized GAL1 in the entire UV spectrum (190−340 nm) was significantly lower than that of GAL1 protein itself (Figure 1B). The result suggests that binding of As(III) to GAL1 also affected the optical behavior of GAL1.

Effect of As(III) Binding on the Electrophoretic Mobility of GAL1. To further understand the biochemical characteristics of the GAL1−As(III) complex, GAL1, GAL1−As(III), and oxidized GAL1 were subjected to mobility shift assays using PAGE and capillary electrophoresis. The results showed that both GAL1 and GAL1−As(III) migrated in the same direction (toward the anode); however, the shift in distance of GAL1−As(III) and oxidized GAL1 was longer than that of GAL1 (Figure 2). Moreover, the mobility of GAL1 also decreased with the concentration of As(III) in a dose-dependent manner as indicated in the capillary electrophoretic profile (Figure 3). These results might suggest that As(III) binding did not alter the net electrostatic charge, but increased the negative charge of GAL1 (the $As(OH)_2O^-$ complex with GAL1). The change in the gel shift pattern also suggests that a conformational change resulted from the binding of As(III) to GAL1.

Microcalorimetric Studies of the Interaction of GAL1 with As(III). To further characterize the thermal dynamics, ITC was used to measure the absorption enthalpy (ΔH_{abs}) between GAL1 and As(III). As shown in Figure 4, the substoichiometric amount of As(III) titration into GAL1 protein was an endothermic reaction with absorption enthalpy (ΔH_{abs}) around 8 to 10 kJ/mol As(III). Binding of As(III)) with sulfhydryl-containing residues in proteins has long been regarded as the basis for the toxic mechanism of As(III). There are several hydrated species of As(III) in solution; however, the predominant form is the pyramidal $As(OH)_3$, which is in equilibrium with the ionized form $As(OH)_2O^-$ (pK_a 9.23). The binding mechanism of As(III) to sulfhydryl groups on GAL1 involved several processes,

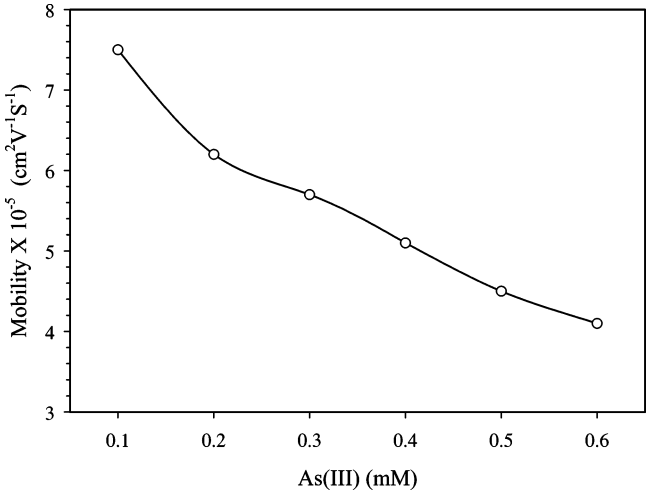

Figure 3. Effect of As(III) on the capillary electrophoretic chromatogram of GAL1. GAL1 (50 μM) was electrophoretically separated in silica capillary in the presence of various concentrations of As(III). After the GAL1 sample was loaded onto the cathode end of the capillary by hydrostatic injection for 5 s, the separation was performed at 20 kV in at least triplicate to ensure reproducibility.

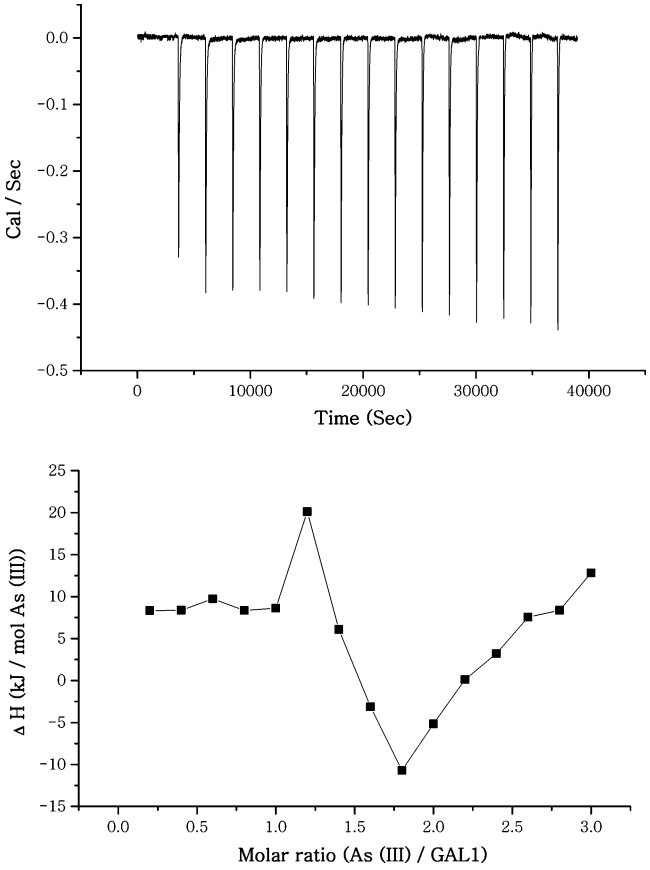

Figure 4. Absorption enthalpies (ΔH_{abs}) of As(III) onto GAL1 in ddH_2O. GAL1 dissolved in 1.4 mL of a ddH_2O solution was loaded into the sample cell and degassed by vacuum. Afterward, the As(III) solution was titrated into the GAL1 solution as described in Experimental Procedures. The output signal was recorded as power (*P*) versus time (*t*) and was integrated and quantified by the amount of adsorbed protein to give the enthalpy change of adsorption (lower panel).

including both endothermic and exothermic processes. The current results indicated that the endothermic enthalpy (breakage of S−H bond in GAL1 and dehydration of As(III)) is higher than the exothermic reaction (As(III)−S bond formation). These results also suggest that the binding of As(III) with GAL1 might

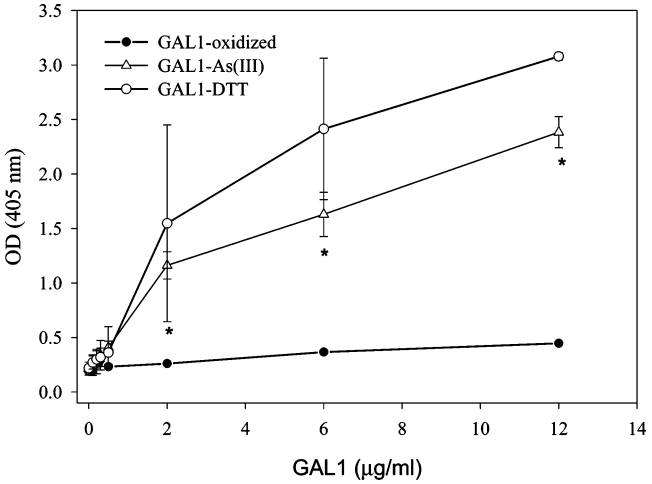

Figure 5. Effect of As(III) on the lectin-binding activity of GAL1. The lectin-binding activities of GAL1 and GAL1−As(III) were determined by a solid-phase binding assay. Various concentrations of GAL1 and GAL1−As(III) were reacted with laminin-coated 96-well plates. Afterward, the wells were incubated with GAL1 monoclonal antibody, followed by incubation with alkaline phosphatase-conjugated anti-mouse Ig. At the end of the incubation, the protein-coated wells were developed with the *p*-nitrophenol phosphate (PNPP) substrate, and color development was monitored at 405 nm as described in Experimental Procedures. Significantly different from control (GAL1-DTT) under the same experimental conditions according to Student's *t*-test. (*) $P < 0.05$.

involve multipoints interactions such as forming specific coordinated compound.

Effect of As(III) Binding on the Lectin-Binding Activity of GAL1. Galectins are a family of proteins that are involved in the regulation of cell adhesion and immune function as well as proliferation and apoptosis (*10*, *31*). GAL1, a member of the galectin family of β-galactoside-binding lectins (*10*), is expressed by many types of normal and neoplastic cells. The biological activities of GAL1 are assumed to depend on its ability to bind β-galactoside. To understand the biological activity of the GAL1−As(III) complex, the lectin-binding activity was measured by a solid-phase binding assay. Results in Figure 5 show that GAL1 bound to lectin in a dose-dependent manner in the presence of dithiothreitol (DTT); in contrast, the lectin-binding activity of oxidized GAL1 was almost completely abolished compared to that of reduced GAL1 (in the presence of DTT). The lectin-binding activity of the GAL1−As(III) complex was slightly lower than that of reduced GAL1, indicating that the binding of As(III) to GAL1 results in a partial loss of the lectin-binding activity of GAL1.

Effect of GAL1 Expression on the Uptake of As(III) by KB Cells. To understand the effect of GAL1 binding on the toxico-dynamics of As(III), we transfected cells with vector control or cDNA encoding hamster GAL1 protein and examined the cellular uptake of As(III) by KB cells. The transfection induced a 6−7-fold increase in GAL1 protein levels compared with sham-transfected cells (KB-V) and parental cells (KB) (data not shown). When cells were treated with various concentrations of As(III) for 24 h, the GAL1-transfected KB cells (KB-GAL1) dose-dependently took up more As(III) compared to KB or empty vector control cells (KB-V) (Figure 6A). Time course experiments also showed that KB-GAL1 cells accumulated more As(III) than did KB or KB-V cells (Figure 6B). Moreover, KB-GAL1 cells were more sensitive to As(III) treatment compared with KB and KB-V cells (Figure 6C). Our previous study also showed that GAL1-transfected 3T3 cells were more vulnerable to As(III) treatment (*22*). Taken together, these results further

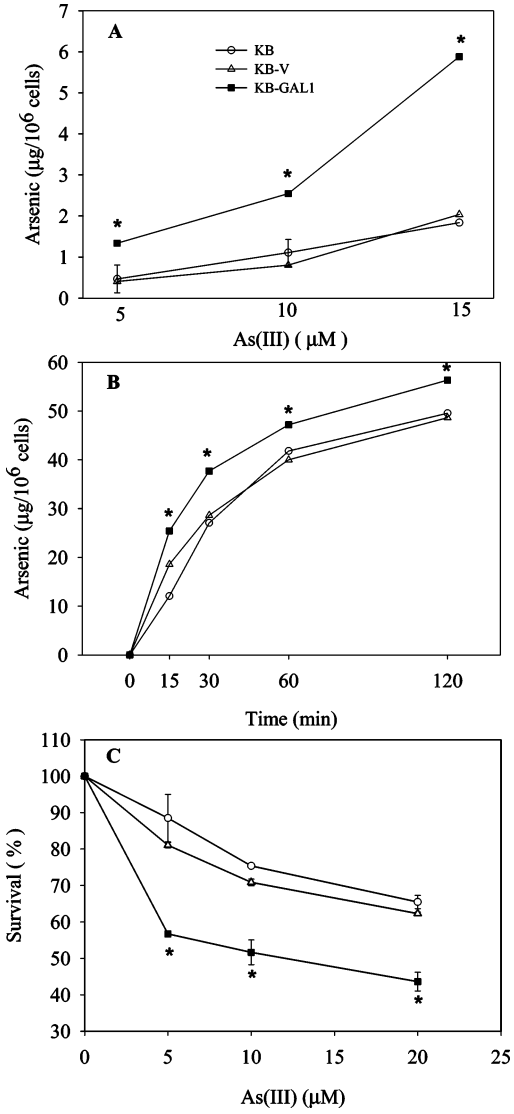

Figure 6. Effect of GAL1-expression on the arsenic efflux (A and B) and toxicity (C) in KB cells. GAL1-transfected KB cells were treated with various concentrations of As(III) for 24 h (A and C) or with 200 μM As(III) for the times indicated (B). At the end of treatment, cells were subjected to determination of arsenic accumulation (A and B) or allowed further growth in drug-free medium for 24 h before subjecting to survival assay (C) as described in Experimental Procedures. Significantly different from control cell (KB and KB-V) under the same experimental conditions according to Student's *t*-test. (*) $P < 0.05$.

suggest that GAL1 overexpression enhanced cellular accumulation and toxicity of As(III).

Discussion

Arsenic is a human carcinogen with no conclusive explanation for its toxic mechanism; however, it is generally believed that the toxicity of arsenic results from its affinity for thiol groups of proteins (*32*). We previously showed that GAL1 is an arsenic-binding protein both in vivo and in vitro (*22*). In this study, we further showed that the binding of As(III) with GAL1 involves a specific coordinated interaction as forming a coordination compound.

The affinity constant (K_d) of As(III) toward GAL1 was around 8.239 ± 2.627 μM as estimated by tryptophan (Trp) fluorescence quenching. Intrinsic tryptophan fluorescence has been used as a probe for determining metal and α-ketoglutarate-dependent dioxygenase binding affinities (*28*). Members of the galectin

family are known to share a well-conserved Trp residue, which plays a crucial role in the binding of sugar in the carbohydrate recognition domain of GAL1 (*20*). Thus, the fluorescence of tryptophan residues in GAL1 may reveal important information regarding the protein conformation, such as the lactose-binding activity. Capillary electrophoresis was also employed to determine the K_d of As(III) toward GAL1 as 3.8 ± 0.8 μM from the point of view of miniaturization, high throughput, high precision, and superior separation efficiency (*33*). Taken together, the K_d values of GAL1 toward As(III) determined by these two methods were higher than those of *N*-acetyllactosamine (0.062 μM) and lactose (0.23 μM) but, however, was lower than that of galactose (22 μM) as estimated by capillary affinophoresis (*30*). The K_d value of GAL1 toward As(III) is also comparable to those of triantennary and tetraantennary asialo type *N*-glycans which were estimated by affinity chromatography (*34*).

Although GAL1 contains six cysteines, it forms a noncovalent homodimer. The six free sulfhydryl groups in GAL1 are critical for its lectin-binding activity and are inactive in the absence of reducing agents (*35*). It has been shown that the oxidized form of GAL1 protein contained three intramolecular disulfide bonds (Cys^2-Cys^{130}, $Cys^{16}-Cys^{88}$, and $Cys^{42}-Cys^{60}$), exhibited distinct axonal regeneration activity, but lacked lectin-binding activity (*25*). The current study also showed that the lectin-binding activity of oxidized GAL1 was completely abolished (Figure 4). As(III) may interact with closely spaced cysteines arranged at 3−6 Å apart on the tertiary structure of a critical cellular protein (*36*). The crystallographic data showed that $Cys^{16}-Cys^{88}$ is separated by 72 amino acids along the GAL1 primary sequence, and they are brought together within 6.75 Å of each other in the folded conformation of the protein. The distances which separate other pairs of thiols are longer than 9.91 Å and are unlikely to be the target for As(III) binding. From this point of view, the potential target of GAL1 for arsenic binding is located at $Cys^{16}-Cys^{88}$ of GAL1. The other four sulfhydryl groups (Cys^2, Cys^{130}, Cys^{42}, and Cys^{60}) of GAL1 remain intact and do not interact with As(III). Therefore, As(III) only interacts with two of the six sulfhydryl groups of GAL1, and the binding of As(III) with $Cys^{16}-Cys^{88}$ would result in partial oxidation of GAL1 proteins. As reported by Hirabayashi and Kasa (*20*), all six site-directed mutants in which one of the cysteine residues has been substituted with serine (C2S, C16S, C42S, C60S, C88S, and C130S) proved to have sugar-binding ability comparable with that of the wild-type GAL1. These results indicate that GAL1 is susceptible to oxidation inactivation possibly through oxidation of all six of its cysteine residues (*37*, *38*); however, substitution of the respective cysteine did not alter the lectin-binding activity of GAL1. These results may explain why the binding of As(III) to GAL1 (in $Cys^{16}-Cys^{88}$ to form the GAL1−As(III) complex) did not result in significant loss of lectin-binding activity.

The effect of As(III) binding on the conformation of GAL1 was also probed by the CD spectrum and a gel mobility shift assay. As reported by Inagaki et al. (*25*) and Pande et al. (*39*), oxidation of GAL1 results in a shift in the CD spectrum. Oxyanion ($As(OH)_2O^-$) binding with GAL1 probably increases the negative charge of GAL1−As(III), and mobility shifts of both CD spectra and gel mobility shift data suggest that As(III) binding results in a conformational change in GAL1. As(III) binding to GAL1 via sulfhydryl groups (in Cys^{16} and Cys^{88}) is likely to cause the conversion of GAL1 to an oxidized form and disturb the normal function of GAL1. Oxidized GAL1 has been shown to promote axonal regeneration (*25*). Our previous study also showed that arsenic exposure results in inhibition of GAL1 expression in Chinese hamster ovary cells (*22*). The down-regulation of GAL1 after exposure to As(III) might help cells avoid the detrimental effects of oxidized GAL1. Moreover, the binding of GAL1 with As(III) also hinders the excretion of As(III) and thus enhances the toxicity of As(III). This assumption was also supported by our results from GAL1-overexpressing KB cells which showed enhanced cellular accumulation of As(III) (Figure 6A,B). Thus, down-regulation of GAL1 may provide advantages for cell survival, as evidenced by the arsenic-resistant cells, SA7 (*22*).

Acknowledgment. We gratefully acknowledge the receipt of financial support from the University System of Taiwan, Joint Research Program - Tsou's Foundation-VGHUST95-P4-10, and National Science Council of Taiwan, Republic of China (NSC93-2311-B-008-002). The authors thank Dan Chamberlin for carefully reading this manuscript.

References

(1) Smith, A. H., Hopenhayn-Rich, C., Bates, M. N., Goeden, H. M., Hertz-Picciotto, I., Duggan, H. M., Wood, R., Kosnett, M. J., and Smith, M. T. (1992) Cancer risks from arsenic in drinking water. *Environ. Health Perspect. 97*, 259−267.

(2) Chen, C. J., and Wang, C. J. (1990) Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. *Cancer Res. 50*, 5470−5474.

(3) Walton, F. S., Harmon, A. W., Paul, D. S., Drobna, Z., Patel, Y. M., and Styblo, M. (2004) Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. *Toxicol. Appl. Pharmacol. 198*, 424−433.

(4) Tseng, C. H. (2004) The potential biological mechanisms of arsenic-induced diabetes mellitus. *Toxicol. Appl. Pharmacol. 197*, 67−83.

(5) Viudez, P., Castano, G., Sookoian, S., Frider, B., and Alvarez, E. (2000) Arsenic and portal hypertension. *Am. J. Gastroenterol. 95*, 1602−1604.

(6) Lee, M. Y., Jung, B. I., Chung, S. M., Bae, O. N., Lee, J. Y., Park, J. D., Yang, J. S., Lee, H., and Chung, J. H. (2003) Arsenic-induced dysfunction in relaxation of blood vessels. *Environ. Health Perspect. 111*, 513−517.

(7) Vahter, M., and Marafante, E. (1985) Reduction and binding of arsenate in marmoset monkeys. *Arch. Toxicol. 57*, 119−124.

(8) Snow, E. T. (1992) Metal carcinogenesis: mechanistic implications. *Pharmacol. Ther. 53*, 31−65.

(9) Kapahi, P., Takahashi, T., Natoli, G., Adams, S. R., Chen, Y., Tsien, R. Y., and Karin, M. (2000) Inhibition of NF-kB activation by arsenite through reaction with a critical cysteine in the activation loop of IkB kinase. *J. Biol. Chem. 275*, 36062−36066.

(10) Barondes, S. H., Cooper, D. N., Gitt, M. A., and Leffler, H. (1994) Galectins. Structure and function of a large family of animal lectins. *J. Biol. Chem. 269*, 20807−20810.

(11) Hirabayashi, J., and Kasai, K. (1993) The family of metazoan metal-independent beta-galactoside-binding lectins: structure, function and molecular evolution. *Glycobiology 3*, 297−304.

(12) Briles, E. B., Gregory, W., Fletcher, P., and Kornfeld, S. (1979) Vertebrate lectins. Comparison of properties of β-galactoside-binding lectins from tissues of calf and chicken. *J. Cell Biol. 81*, 528−537.

(13) Zhou, Q., and Cummings, R. D. (1993) L-14 lectin recognition of laminin and its promotion of in vitro cell adhesion. *Arch. Biochem. Biophys. 300*, 6−17.

(14) Cooper, D. N., Massa, S. M., and Barondes, S. H. (1991) Endogenous muscle lectin inhibits myoblast adhesion to laminin. *J. Cell Biol. 115*, 1437−1448.

(15) Cerra, R. F., Haywood-Reid, P. L., and Barondes, S. H. (1984) Endogenous mammalian lectin localized extracellularly in lung elastic fibers. *J. Cell Biol. 98*, 1580−1589.

(16) Cooper, D. N., and Barondes, S. H. (1990) Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. *J. Cell Biol. 110*, 1681−1691.

(17) Cleves, A. E., Cooper, D. N., Barondes, S. H., and Kelly, R. B. (1996) A new pathway for protein export in *Saccharomyces cerevisiae*. *J. Cell Biol. 133*, 1017−1026.

(18) Cavigelli, M., Li, W. W., Lin, A., Su, B., Yoshioka, K., and Karin, M. (1996) The tumor promotor arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. *EMBO J. 15*, 6269−6279.

(19) Liao, D. I., Kapadia, G., Ahmed, H., Vasta, G. R., and Herzberg, O. (1994) Structure of S-lectin, a developmentally regulated vertebrate β-galactoside-binding protein. *Proc. Natl. Acad. Sci. U.S.A. 91*, 1428−1432.

(20) Hirabayashi, J., and Kasai, K. (1991) Effect of amino acid substitution by sited-directed mutagenesis on the carbohydrate recognition and stability of human 14-kDa β-galactoside-binding lectin. *J. Biol. Chem. 266*, 23648−23653.
(21) Barondes, S. H. (1984) Soluble lectins: a new class of extracellular proteins. *Science 223*, 1259−1264.
(22) Chang, K. N., Lee, T. C., Tam, M. F., Chen, Y. C., Lee, L. W., Lee, S. Y., Lin, P. J., and Huang, R. N. (2003) Identification of galectin I and thioredoxin peroxidase II as two arsenic binding proteins in Chinese hamster ovary cells. *Biochem. J. 371*, 495−503.
(23) Huang, R. N., and Lee, T. C. (1996) Cellular uptake of trivalent arsenite and pentavalent arsenate in KB cells cultured in phosphate-free medium. *Toxicol. Appl. Pharmacol. 136*, 243−249.
(24) Mazurek, N., Conklin, J., Byrd, J. C., Raz, A., and Bresalier, R. S. (2000) Phosphorylation of the β-galactoside-binding protein galectin-3 modulates binding to its ligands. *J. Biol. Chem. 275*, 36311−36315.
(25) Inagaki, Y., Sohma, Y., Horie, H., Nozawa, R., and Kadoya, T. (2000) Oxidized galectin-1 promotes axonal regeneration in peripheral nerves but does not possess lectin properties. *Eur. J. Biochem. 267*, 2955−2964.
(26) Lin, F. Y., Chen, C. S., Chen, W. Y., and Yamamoto, S. (2001) Microcalorimetric studies of the interaction mechanisms between proteins and Q-sepharose at pH near the isoelectric point (pI): effects of NaCl concentratrion, pH value, and temperature. *J. Chromatogr., A 912*, 281−289.
(27) Iglesias, M. M., Elola, M. T., Martinez, V., Fink, N., and Wolfenstein-Todel, C. (2003) Identification of an equilibrium intermediate in the unfolding process of galectin-1, which retains its carbohydrate-binding specificity. *Biochim. Biophys. Acta 1648*, 164−173.
(28) Dunning Hotopp, J. C., Auchtung, T. A., Hogan, D. A., and Hausinger, R. P. (2003) Intrinsic tryptophan fluorescence as a probe of metal and alpha-ketoglutarate binding to TfdA, a mononuclear non-heme iron dioxygenase. *J. Inorg. Biochem. 93*, 66−70.
(29) Rundlett, K. L., and Armstrong, D. W. (1996) Examination of the origin, variation, and proper use of expressions for the estimation of association constants by capillary electrophoresis. *J. Chromatogr., A 721*, 173−186.
(30) Shimura, K., Arata, Y., Uchiyama, N., Hirabayashi, J., and Kasai, K. (2002) Determination of the affinity constant of recombinant human galectin-1 and -3 for simple saccharides by capillary affinophoresis. *J. Chromatogr., B 768*, 199−210.
(31) Jessell, T. M., Hynes, M. A., and Dodd, J. (1990) Carbohydrates and carbohydrate-binding proteins in the nervous system. *Annu. Rev. Neurosci. 13*, 227−255.
(32) Sunderman, F. W. (1979) Mechanism of metal carcinogens. *Biol. Trace Elem. Res. 1*, 53−58.
(33) Duijin, R. M. G., Frank, J., van Dedem, G. W. K., and Baltussen, E. (2000) Recent advances in affinity capillary electrophoresis. *Electrophoresis 21*, 3905−3918.
(34) Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, T., Urashima, T., Oka, T., Futai, M., Muller, W. E. G., Yagi, F., and Kasai, K. (2002) Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. *Biochim. Biophys. Acta 1572*, 232−254.
(35) Abbott, W. M., and Feizi, T. (1991) Soluble 14-kDa beta-galactoside-specific bovine lectin. Evidence from mutagenesis and proteolysis that almost the complete polypeptide chain is necessary for integrity of the carbohydrate recognition domain. *J. Biol. Chem. 266*, 5552−5557.
(36) Bhattacharjee, H., and Rosen, B. P. (1996) Spatial proximity of Cys113, Cys172, and Cys422 in the metalloactivation domain of the ArsA ATPase. *J. Biol. Chem. 271*, 24465−24470.
(37) Tracy, B. M., Feizi, T., Abbott, W. M., Carruthers, R. A., Green, B. N., and Lawson, A. M. (1992) Subunit molecular mass assignment of 14,654 Da to the soluble β-galactoside-binding lectin from bovine heart muscle and demonstration of intramolecular disulfide bonding associated with oxidative inactivation. *J. Biol. Chem. 267*, 10342−10347.
(38) Whitney, P. L., Powell, J. T., and Sanford, G. L. (1986) Oxidation and chemical modification of lung β-galactoside-specific lectin. *Biochem. J. 238*, 683−689.
(39) Pande, A. H., Gupta, R. K., Sumati, and Hajela, K. (2003) Oxidation of goat hepatic galectin-1 induces change in secondary structure. *Protein Pept. Lett. 10*, 265−275.

TX0503348