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Mammalian glucose permease GLUT1 facilitates transport of arsenic trioxide and methylarsonous acid†

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Abstract

Arsenic exposure is associated with hypertension, diabetes and cancer. Some mammals methylate arsenic. *Saccharomyces cerevisiae* hexose permeases catalyze $As(OH)_3$ uptake. Here we report that mammalian glucose transporter GLUT1 catalyzes $As(OH)_3$ and $CH_3As(OH)_2$ uptake in yeast or in *Xenopus laevis* öocytes. Expression of GLUT1 in a yeast lacking other glucose transporters allows for growth on glucose. Yeast expressing yeast HXT1 or rat GLUT1 transport $As(OH)_3$ and $CH_3As(OH)_2$. The K_m of GLUT1 is to 1.2 mM for $CH_3As(OH)_2$, compared to a K_m of 3 mM for glucose. Inhibition between glucose and $CH_3As(OH)_2$ is noncompetitive, suggesting differences between the translocation pathways of hexoses and arsenicals. Both human and rat GLUT1 catalyze uptake of both $As(OH)_3$ and $CH_3As(OH)_2$ in öocytes. Thus GLUT1 may be a major pathway uptake of both inorganic and methylated arsenicals in erythrocytes or the epithelial cells of the blood-brain barrier, contributing to arsenic-related cardiovascular problems and neurotoxicity.

Arsenic ranks first on the United States Government's Comprehensive Environmental Response, Compensation, and Liability (Superfund) Act Priority List of Hazardous Substances <http://www.atsdr.cdc.gov/cercla/05list.html>. In response to the Safe Water Drinking Act, the United States Environmental Protection Agency has set the allowable level of arsenic in drinking water at 10 ppb <http://www.epa.gov/safewater/arsenic/regulations.html>, which is less than the level in the water supplies of many U.S. municipalities <http://water.usgs.gov/nawqa/trace/arsenic/>. In addition, the Environmental Protection Agency's Office of Pesticide Programs is concerned with exposure to the organic arsenicals methylarsenic acid and dimethylarsenic acid, pentavalent arsenicals used as pesticides and herbicides <http://www.epa.gov/sab/pdf/arsenic_review_panel_final_charge_7-25-05.pdf>.

Health effects associated with arsenic exposure include cardiovascular and peripheral vascular disease, neurological disorders, diabetes mellitus and various cancers, including liver, bladder, kidney and skin [1-3]. Arsenic trioxide, which is As_2O_3 in the solid, anhydrous form and $As(OH)_3$ in solution at physiological pH [4], is used clinically as a chemotherapeutic drug for treatment of acute promyelocytic leukemia [5]. Although epidemiological studies demonstrated that arsenic exposure is associated with a high frequency of circulatory and neurological problems [2,6,7], how arsenic causes non-cancer-related diseases is far from clear.

†Abbreviations: deoxyglucose (DOG).

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Humans and some other mammals metabolize inorganic arsenic to a variety of methylated species that may be more toxic than their inorganic counterparts [8], These inorganic and organic species are found in many tissues and are excreted in urine and feces [9,10]. In general, trivalent arsenicals are more toxic than penvalent, and methylated trivalent arsenicals are more cytotoxic than inorganic $As(OH)_3$ [11].

We have previously shown that some aquaglyceroporins (AQPs) facilitate $As(OH)_3$ movement, including mammalian AQP7 and AQP9 [12,13]. AQP9 also conducts methylarsonous acid or $CH_3As(OH)_2$ [14], an intermediate in the pathway of arsenic methylation. AQP7 is expressed primarily in kidney, testis and adipose tissue [15,16], and AQP9 is expressed primarily in liver and astrocytes [17]. How are $As(OH)_3$ and/or $CH_3As(OH)_2$ transported into cells that do not have AQP7 or AQP9 such as erythrocytes and the epithelial cells that form the blood-brain barrier? *S. cerevisiae* hexose transporters, members of the major facilitator superfamily, catalyze uptake of $As(OH)_3$ [18]. The mammalian homologue GLUT1, which is found in erythrocytes and the epithelial cells that form the blood-brain barrier, mediates the majority uptake of glucose into brain through the blood-brain barrier [19]. GLUT1-deficiency syndrome (GLUT1DS), which results in an inadequate energy supply to brain, manifests as delayed neurological development and other neurological problems [20,21].

Here we report that GLUT1 facilitates uptake of $As(OH)_3$ and $CH_3As(OH)_2$ using heterologous expression in *S. cerevisiae* and *X. laevis* öocytes. *S. cerevisiae* is a valuable model system for structure-function studies of mammalian membrane proteins such as GLUT1 [22,23]. A yeast strain with low uptake of $As(OH)_3$ and $CH_3As(OH)_2$ was constructed by deletion of all 18 hexose transporters, *FPS1*, which encodes an aquaglyceroporin that facilitates $As(OH)_3$ influx, and *ACR3*, the gene for an $As(OH)_3$ efflux carrier protein. This strain is unable to grow on glucose as a carbon source. Expression of the gene for a rat GLUT1 variant [24] allowed for growth on glucose. In this strain GLUT1 mediated low rates of $As(OH)_3$ uptake and higher rates of $CH_3As(OH)_2$ uptake. Uptake of glucose was inhibited noncompetitively by $CH_3As(OH)_2$, and inhibition of $CH_3As(OH)_2$ uptake by glucose was noncompetitive. In öocytes expressing either human or rat GLUT1, $As(OH)_3$ uptake was lower than $CH_3As(OH)_2$, consistent with GLUT1 catalyzing faster uptake of $CH_3As(OH)_2$ than $As(OH)_3$. Since GLUT1 is more widely distributed than AQP7 and AQP9, transport of inorganic and organic arsenicals by GLUT1 may represent the major pathway for entry of arsenic into cells and organs such as erythrocytes and brain.

Experimental Procedures

Strains and plasmids

E. coli strain DH-5α (*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi (lac-proAB) F′ (traD36 proAB+ lacIq lacZ.M15*) was used for molecular cloning. *S. cerevisiae* strain EBY.VW1000, in which all 18 hexose permeases were deleted [25] was used to construct strain HD300 (*fps1::leu acr3:his*) by a step-wise replacement of 0.8 kbp of *FPS1* with a *LEU2* gene and 1.15 kbp of *ACR3* with a *HIS3* gene. Gene disruptions were verified by both polymerase chain reaction and phenotypic analysis of arsenite resistance. HD300 was transformed with pTHHXT series plasmids carrying *HXT* genes [18] or plasmid YEpH2-rGLUT1, which carries a V69M variant of GLUT1 under control of the yeast *HXT2*promoter [24]. The V69M mutation has no affect on glucose transport but allows for growth of yeast on glucose as a carbon source. Plasmid pL2-5-GLUT1 was constructed by amplification of the rat V69M GLUT1 gene using a pair of primers 5′-GAGATCTATGGAGCCCAGCAGCAAGAAG-3′ (*Bgl*II site underlined) and 5′-GAGATCTTCACACTTGGGAGTCAGCCC-3′ (*Bgl*II site underlined). The GLUT1 gene was cloned into pGEMT-easy (Promega, Madison, WI), and digested with *Bgl*II. The 1.5 kbp *Bgl*II fragment was cloned into *Bgl*II-digested pL2-5 [26], resulting in pL2-5-GLUT1. The

sequence of the GLUT1 gene was verified by DNA sequencing. Human GLUT1 was cloned in a same strategy by using a pair of primers, 5′ GAGATCTATGGAGCCCAGCAGCAAGAAG (*Bgl*II site underlined) and 3′ GAGATCTTCACACTTGGGAATCAGC (*Bgl*II site underlined), generating pL2-5-hGLUT1.

Media

S. cerevisiae was grown at 30° C in minimal SD [27] medium with either 2% glucose or 2% maltose as carbon source supplemented with auxotrophic requirements. *E. coli* was grown in Luria-Bertani medium [28] supplemented when appropriate with 125 μg/ml ampicillin.

DNA manipulations

Molecular methods were carried out as described [28]. Transformation of yeast cells was carried out using a Geno easy-transform kit (Geno Technologies, St. Louis, MO). Yeast genomic DNA was isolated using QIAamp spin column (Qiagen Inc.).

Transport assays

Transport of $As(OH)_3$ or $CH_3As(OH)_2$ in both yeast and öocytes was assayed as described previously [14]. Glucose uptake in yeast cells was assayed using D-[U-^{14}C]glucose (PelkinElmer, Wellesley, MA), as described [29]. Uptake of [^{3}H]2-deoxyglucose (DOG) (2 μCi/ml) (PelkinElmer, Wellesley, MA) in öocytes was measured as described [30].

Results

GLUT1 catalyzes uptake of both $As(OH)_3$ and $CH_3As(OH)_2$

GLUT1 activity by rat GLUT1 was assayed in *S. cerevisiae* strain HD300. A rat V69M rGLUT1 variant under control of the yeast *HXT*2 promoter allows for better growth than wild type rGLUT1 with glucose as a carbon source, and yeast cells expressing this rGLUT1 take up glucose [24], so subsequent assays were conducted with V69M. Strain HD300 was constructed from strain EBY.VW1000, in which the genes for eighteen hexose transporters were deleted [24], by subsequent deletion of *FPS1* [14] and *ACR3* [31,32].

The ability of rGLUT1 to transport $As(OH)_3$ and $CH_3As(OH)_2$ was compared with yeast permeases Hxt1p, Hxt3p, Hxt4p, Hxt5p, Hxt7p, Hxt9p and Hxt10p. Each catalyzed $As(OH)_3$ transport (Fig. 1A). Hxt7p gave the highest rate of $As(OH)_3$ uptake, while rGLUT1 exhibited low uptake at 0.1 mM sodium arsenite. Importantly, hexose transporters also catalyzed uptake of $CH_3As(OH)_2$ at 50 μM at rates up to 50-fold higher than $As(OH)_3$. Their relative efficiencies were reversed compared with $As(OH)_3$: rGLUT1 had the highest rate of uptake, while Hxt7p gave the lowest (Fig. 1B). Hexose permeases have different affinities, so the observed rank order may not apply at all concentrations of substrates. $CH_3As(OH)_2$ uptake by the yeast hexose transporters was inhibited by glucose (Fig. 1C).

Kinetic analysis of rGLUT1

The affinity of rGLUT1 for $CH_3As(OH)_2$, with a K_m of 1.2 mM (Fig. 2A), is similar to that for glucose, which is approximately 3 mM when assayed in yeast [24]. rGLUT1 appears to be selective for $CH_3As(OH)_2$; the rate of $As(OH)_3$ uptake by rGLUT1 was so much lower than the methylated species that it was not possible to determine the kinetic constants for $As(OH)_3$ uptake by rGLUT1. Uptake of $CH_3As(OH)_2$ by rGLUT1 is inhibited by glucose noncompetitively (Fig. 2B), and glucose uptake by rGLUT1 is inhibited by $CH_3As(OH)_2$ noncompetitively (Fig. 2C). These results suggest that the two substrates may utilize different initial binding sites or different translocation pathways.

$CH_3As(OH)_2$ transport by rGLUT1 and HXT1 is inhibited by hexoses but not cytocholasin B or forskolin

Substrates for rGLUT1 include glucose, galactose and mannose but not fructose [33]. Expression of rGLUT1 in yeast strain HD300 enables cells to use glucose, galactose and mannose as carbon sources, but cells cannot grow on fructose as sole carbon source (Fig. 3A), and $CH_3As(OH)_2$ uptake in those cells was inhibited by glucose, mannose but not galactose, fructose or glycerol (Fig. 3C). By way of comparison, yeast cells with Hxt1p transport glucose, mannose and fructose but not galactose [34] (Fig. 3B). Cells expressing *HXT1* utilize glucose, mannose and fructose but not galactose as sole carbon sources (Fig. 3A), and $CH_3As(OH)_2$ uptake in those cells was inhibited by glucose, mannose and fructose but not galactose or glycerol (Fig. 3C). The lack of inhibition of GLUT1-mediated $CH_3As(OH)_2$ uptake by galactose suggests that the initial binding sites for arsenicals and hexoses might be different, consistent with the reciprocal noncompetitive inhibition between glucose and $CH_3As(OH)_2$. Cytochalasin B and forskolin, inhibitors of mammalian glucose permeases but not yeast hexose transporters [35,36], inhibited rGLUT1-mediated uptake of glucose but not $CH_3As(OH)_2$ (data not shown), further indicating that there are differences in the way that hexoses and $CH_3As(OH)_2$ are recognized by rGLUT1.

Both human and rat GLUT1 catalyze $CH_3As(OH)_2$ transport in öocytes

Although the rat V69M GLUT1 expresses in yeast, normal human GLUT1 is not expressed well enough to allow for characterization in yeast. To directly compare human and rat GLUT1, their ability to increase permeability to glucose and $CH_3As(OH)_2$ was examined in *X. laevis* öocytes (Fig. 4). Both human and rat V69M variant GLUT1 cRNAs were prepared in vitro and microinjected into öocytes. Expression of the two GLUT1s was estimated by immunoblotting using commercial antibody (Alpha Diagnosis), and both appear to be expressed at similar levels (data not shown). Human and rat GLUT1 transported 2-deoxyglucose at similar rates (Fig. 4A), but rGLUT1 transported $CH_3As(OH)_2$ at faster rates than hGLUT1 (Fig. 4B). Consistent with the results obtained with yeast expression, rat GLUT1 transported $CH_3As(OH)_2$ much faster than $As(OH)_3$, which was barely above the rate of water-injected öocytes (data not shown), demonstrating that GLUT1 is a much better $CH_3As(OH)_2$ transporter than $As(OH)_3$ transporter.

Discussion

We have shown previously that inorganic trivalent arsenic, $As(OH)_3$, is transported by mammalian aquaglyceroporin channels such as human and rat AQP7 and AQP9 [12,13]. AQP9 also facilitates movement of the monomethylate species, $CH_3As(OH)_2$ [14]. AQP7 is found predominately in kidney, testis and adiopose tissue [37,38], and AQP9 is found mainly in liver, spleen and brain [39] but not in the epithelial cells the form the blood-brain barrier [40]. This raises the question of how tissues that do not have detectable aquaglyceroporins, including heart and the blood-brain barrier, take up trivalent arsenicals. Glucose transporter isoform 1 or GLUT1, a homologue of the yeast Hxt permeases, is widely distributed in mammalian tissues. It is the major pathway for glucose uptake in many cell types, including neonatal heart, erythrocytes and the endothelial cells that form the blood–brain barrier. Although adult heart does not depend on glucose for energy, in neonatal heart, glucose utilization via GLUT1 is a major source of energy [41]. Rat GLUT1 can be functionally expressed in yeast [24], which allowed analysis of its arsenic transport properties. Although human GLUT1 does not express well in yeast, it is reasonable to assume that its transport properties will be essentially the same as rGLUT1 since rGLUT1 and hGLU1 are 97% identical. Compared with a number of the yeast hexose transporters, rGLUT1 does not transport $As(OH)_3$ efficiently (Fig. 1A). In contrast, rGLUT1 has the highest rate of $CH_3As(OH)_2$ uptake at 50 μM, followed yeast Hxt1p, Hxt3p, Hxt4p, Hxt5p, Hxt9p and Hxt10p (Fig. 1B). Importantly, for each transport protein,

the rate of uptake of $CH_3As(OH)_2$ was at least an order of magnitude higher than for inorganic trivalent arsenic, demonstrating selectivity for the organic arsenical. These results suggest that uptake of $CH_3As(OH)_2$ might be common property of members of the eukaryotic hexose permease family. Transport could be a major contributor to the adverse effects of arsenic in mammalian cells, especially - as if often the case - uptake is the rate limiting step for subsequent intracellular reactions.

Glucose permeases undoubtedly transport $As(OH)_3$ and $CH_3As(OH)_2$ adventitiously. It is not intuitively obvious why a trivalent metalloid and a sugar should be recognized by the same transporter but the observation of transport of both species suggests that they may have chemical or structural similarities. We have proposed that trimer forms a six-membered cyclic oxo-bridged ring which is sufficiently glucose-like to be recognized by yeast hexose transporters [18]. While there is no structural information on $CH_3As(OH)_2$, it is reasonable to assume that it can also form a trimeric cyclic glucose-like ring structure. These ring forms of $As(OH)_3$ and $CH_3As(OH)_2$ may compete for the same binding site on the glucose permeases and share the translocation pathway. Another possibility is that they are bound as monomers of $As(OH)_3$ or $CH_3As(OH)_2$, with the binding site on the glucose permeases filled with one, two or three molecules. GLUT1 has been shown to function as a water channel [42], and a T310I GLUT1 mutant, identified in a patient with GLUT1-deficiency syndrome, has reduced glucose transport and increased water permeation that has lost its inhibition by glucose, suggesting that water goes through a different pathway in GLUT1 than glucose [43]. Thus, just as aquaporin channels conduct $As(OH)_3$ or $CH_3As(OH)_2$, these arsenicals might also move through a water channel in GLUT1. This is supported by two lines of evidence. First, $CH_3As(OH)_2$ inhibits glucose transport noncompetitively, and vice versa, which would be expected if they did not bind at the same site. Second, the classical inhibitors of glucose transport cytochalsin B and forskolin, do not inhibit $CH_3As(OH)_2$ uptake. Future experiments such as mutant analysis will be designed to test these possibilities.

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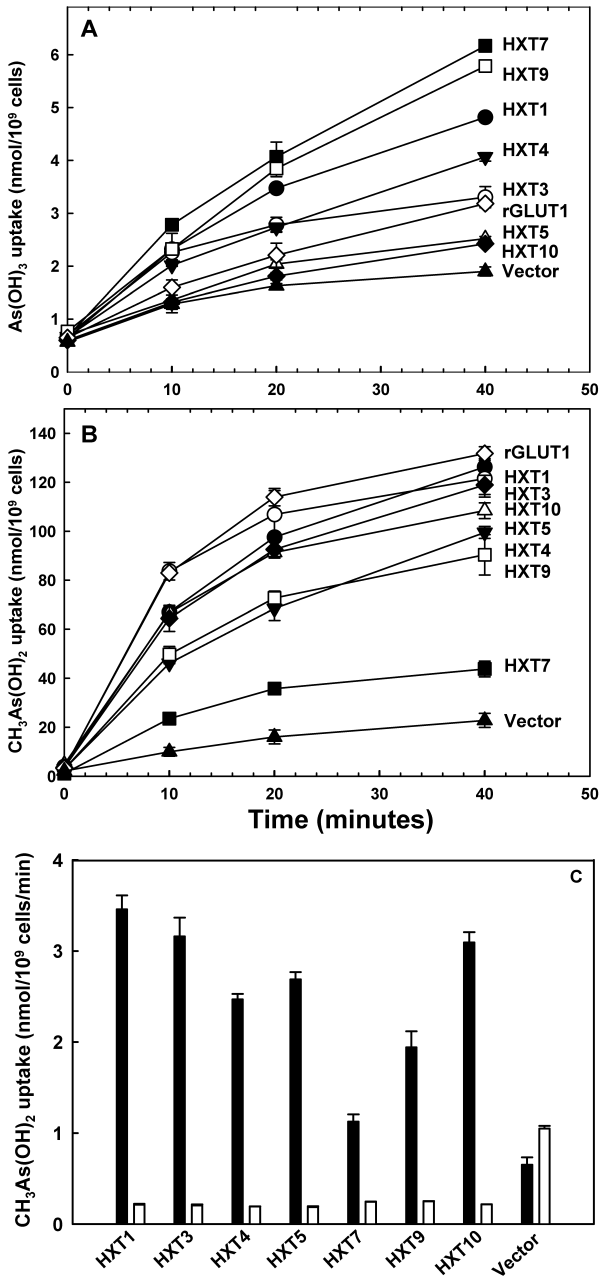

Fig. 1.

***HXT*s and rGLUT1 facilitate uptake of $As(OH)_3$ and $CH_3As(OH)_2$ in *S. cerevisiae*.** Yeast cells expressing hexose transport genes ((▲), vector; (◇),YEpH2-rGLUT1; (●);pTHHXT1; (○),pTHHXT3; (▼),pTHHXT4; (△), pTHHXT5; (■),pTHHXT7; (□),pTHHXT9; (◆), pTHHXT10) were assayed for uptake of 0.1 mM $As(OH)_3$ (A) or 50 μM $CH_3As(OH)_2$ (B), as described under Experimental Procedures. (C) Inhibition of 50 μM $CH_3As(OH)_2$ uptake by 50 mM glucose was assayed after 30 min. The values in each plot are the mean of three independent assays. The error bars represent the standard deviation of the mean calculated using SigmaPlot 9.0.

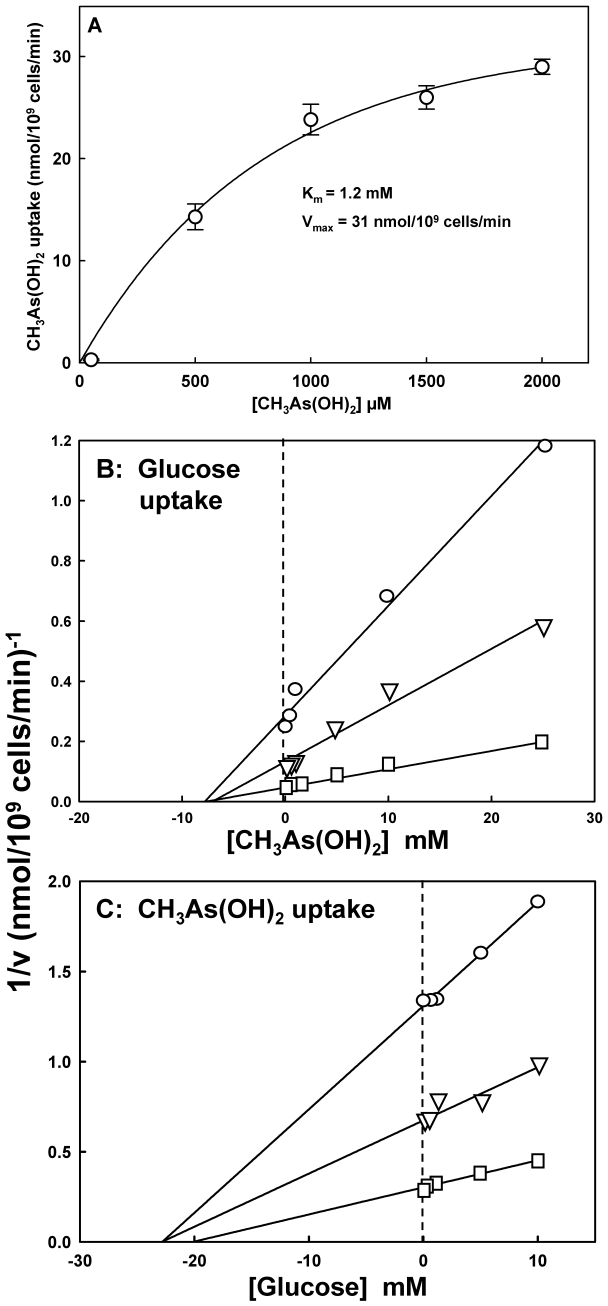

Fig. 2.

Kinetic analysis of rGLUT1. A. Kinetics of $CH_3As(OH)_2$ uptake. Kinetic parameters were calculated from the rates of uptake in yeast strain HD300 YEpH2-rGLUT1 at the indicated concentrations of $CH_3As(OH)_2$ after subtraction of the rates from strain HD300 alone, which lacks hexose transport genes. **B.** $CH_3As(OH)_2$ noncompetitively inhibits glucose uptake. Uptake of [^{14}C]glucose in HD300 YEpH2-rGLUT1 was assayed for 30 sec with the indicated concentrations of $CH_3As(OH)_2$ at (□), 50 µM; (▽), 100 µM; or (○), 250 µM glucose. $CH_3As(OH)_2$ was added 3 min prior to the start of each assay at room temperature. [^{14}C]glucose was added at a final concentration of 1mM. **C.** Glucose noncompetitively inhibits $CH_3As(OH)_2$ uptake. Uptake of $CH_3As(OH)_2$ in HD300 YEpH2-rGLUT1 was assayed for 30 sec with the

indicated concentrations of glucose at (□), 50 μM; (∇), 100 μM; or (○), 250 μM $CH_3As(OH)_2$. Glucose was added 3 min prior to the start of each assay at room temperature. $CH_3As(OH)_2$ was added at a final concentration of 1mM. The values in each plot are the mean of three independent assays. The error bars represent the standard deviation of the mean calculated using SigmaPlot 9.0.

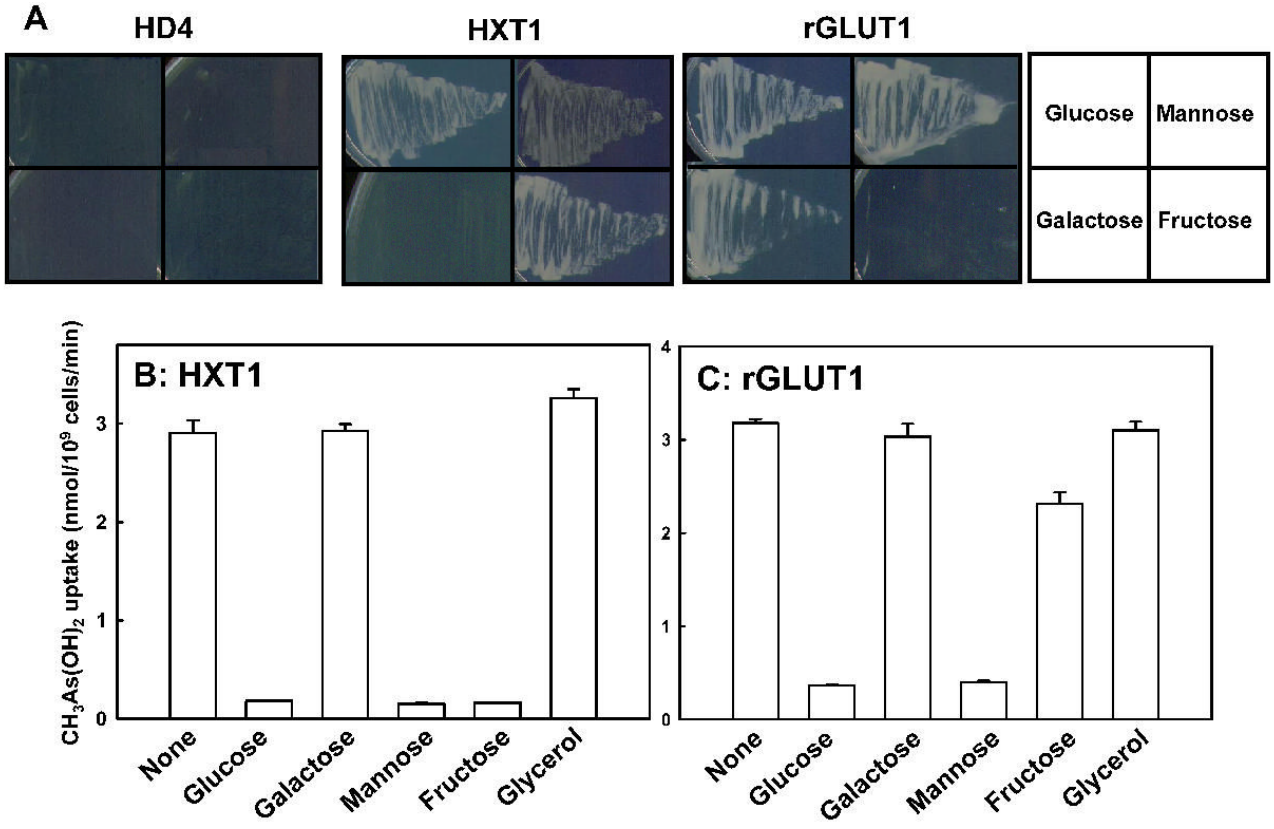

Fig. 3.

$CH_3As(OH)_2$ transport by either rGLUT1 or HXT1 is inhibited by hexoses. A. Growth of yeast strain HD300 YEpH2-rGLUT1 (right), pTHHXT1 (middle) or no hexose permease genes (left) in SD minimal medium supplied with 2% of the glucose, mannose, galactose or fructose, as indicated. **B.** Inhibition of 50 μM $CH_3As(OH)_2$ transport in HD300 pTHHXT1 by the indicated hexoses, each at 50 mM. **C.** Inhibition of $CH_3As(OH)_2$ transport in strain HD300 YEpH2-rGLUT1 by the indicated hexoses, each at 50 mM. The values in each plot are the mean of three independent assays.

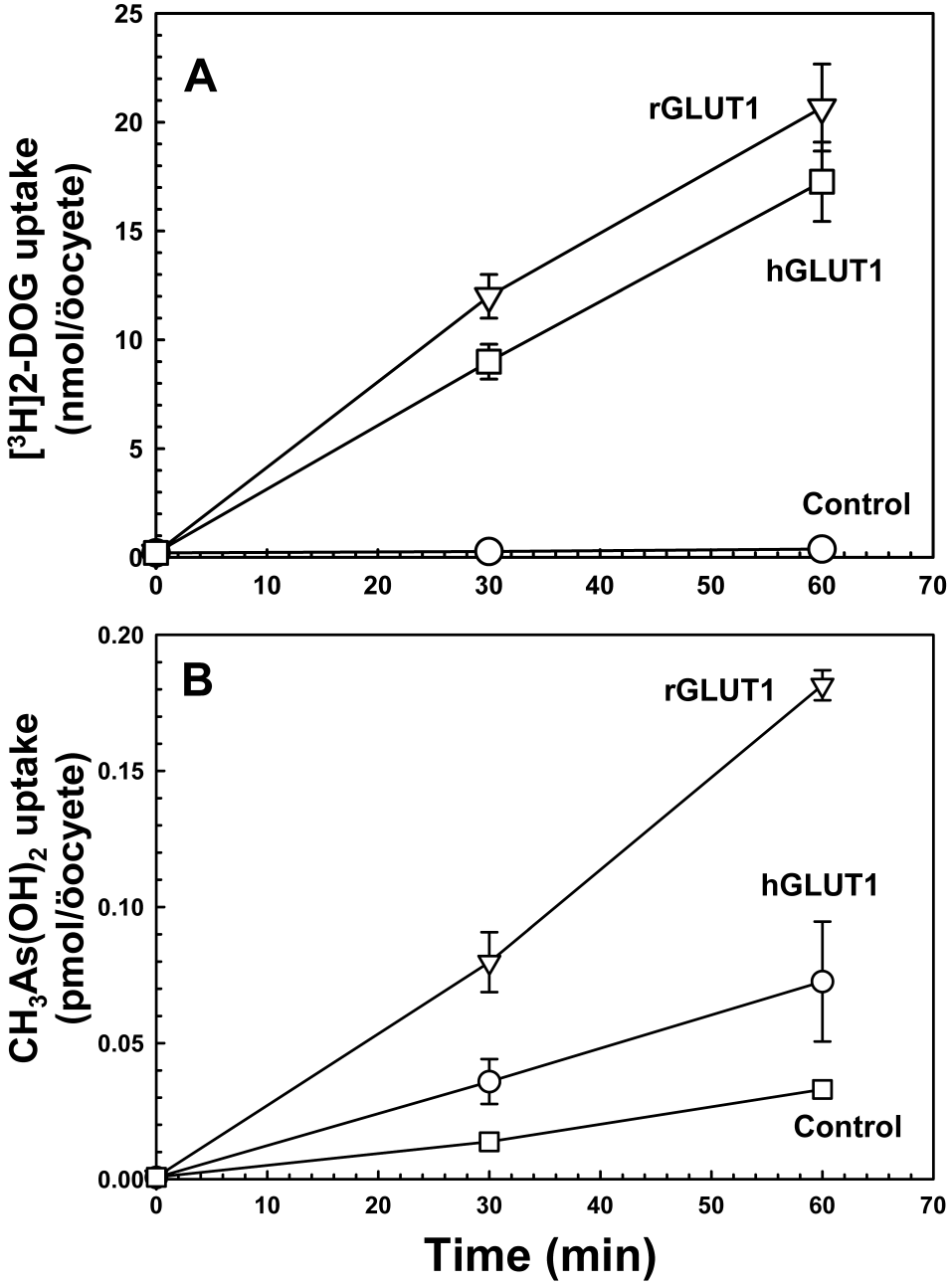

Fig. 4.

GLUT1 facilitates uptake of $CH_3As(OH)_2$ in *X. laevis* öocytes. A: Transport of 1 mM [^{3}H] deoxyglucose in öocytes injected with rGLUT1 cRNA (▽), hGLUT1 cRNA (○) or water injected (□). **B:** Transport of 0.1 mM $CH_3As(OH)_2$ in öocytes injected with rGLUT1 cRNA (▽), hGLUT1 cRNA (○) or water injected (□). The values in each plot are the mean of three independent assays.