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ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · AUGUST 2003

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Effect of pH on Mercury Uptake by an Aquatic Bacterium: Implications for Hg Cycling

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We studied the effect of increasing hydrogen ion (H^+) concentration on the uptake of mercury (Hg(II)) by an aquatic bacterium. Even small changes in pH (7.3−6.3) resulted in large increases in Hg(II) uptake, in defined media. The increased rate of bioaccumulation was directly proportional to the concentration of H^+ and could not be explained by assuming that the source of Hg to the bacteria was diffusion of neutrally charged species such as $HgCl_2$. Thus, pH appeared to affect a facilitated mechanism by which Hg(II) is taken up by the cells. Lowering the pH of Hg solutions mixed together with natural dissolved organic carbon, or with whole lake water, also increased bacterial uptake of Hg(II). These findings have several potential implications for mercury cycling, including effects on elemental mercury production, mercury sedimentation, and microbial methylation of Hg(II), and could be part of the explanation for the observed positive correlation between lake acidity and methyl mercury levels in fish.

Introduction

In freshwater lakes, one of the best predictors of fish mercury concentration is lake water pH, with fish in low pH lakes having higher mercury concentration than fish in higher pH lakes (e.g., refs *1* and *2*). Mercury (Hg) in fish is primarily in the form of methyl mercury (MeHg (*3*)).

One explanation for the higher fish MeHg concentrations at low pH is that methylation of Hg(II) in lake water and sediment surfaces is enhanced in acidified lakes (*4*−*6*). A plausible reason for this enhanced methylation could be enhanced uptake of Hg(II) by the methylating bacteria at lower pH. Uptake could be stimulated either by changes in the chemical speciation of Hg(II) or by an effect on the bacterial uptake process itself. With respect to chemical speciation, dissolved organic carbon (DOC) in natural waters is less negatively charged at low pH and therefore less likely to complex Hg(II), making it more available to the bacteria (*7*, *8*). With respect to effects on the uptake process itself, there is recent evidence for facilitated uptake of Hg(II) by bacteria, which are capable of accessing both charged and uncharged Hg(II) species (*9*). Changes in pH are known to affect rates of facilitated uptake of metals other than mercury (*10*), and this might also be the case for facilitated uptake of mercury.

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We initiated a study on the effects of pH on Hg(II) uptake using a Hg(II) specific bioreporter, *Vibrio anguillarum* pRB28. This is a genetically engineered strain of an aquatic bacterial species that produces light in an amount that is directly proportional to the amount of Hg(II) that crosses the bacterial membrane (*9*). The *V. anguillarum* strain was transformed with the *mer-lux* bioreporter plasmid pRB28 (*11*). In this plasmid, the expression of the bacterial *lux* genes, for light production, is controlled by the same regulatory system that controls expression of the genes for Hg(II) reductase in mercury resistant bacteria, but in the *mer-lux* plasmid the genes for the reductase have been removed and replaced with genes for light production. This system only responds to Hg(II) that has entered cells, and so is an excellent reporter system for Hg(II) uptake.

In our study, using both defined inorganic solutions and lake water, uptake of Hg(II) was faster at lower pH, and the increased rate of uptake was not related to changes in neutral Hg species such as $HgCl_2$ or $Hg(OH)_2$. Rather, uptake of both charged and uncharged Hg(II) species appeared to increase as H^+ increased, indicating a facilitated bacterial Hg(II) uptake process that responds to pH. This could have a number of consequences for Hg cycling, such as effects on loss of elemental Hg from the surface of lakes and production of methyl mercury in surface sediments.

Materials and Methods

Bacterial Strains. Bacterial strains used in this study were *Vibrio anguillarum* (ATCC 14181) pRB28 and *V. anguillarum* pRB27 and are described in Golding et al. (*9*). *V. anguillarum* is an aquatic species capable of inhabiting both freshwater and marine environments and can grow under either aerobic or anaerobic conditions. In the pRB28 strain, light production is dependent on and proportional to Hg(II) uptake, while in the pRB27 strain, light production is constitutive (*8*).

Clean Protocol. Culture flasks, test tubes, Teflon centrifuge tubes, reagent storage bottles, and spectrophotometer tubes were acid washed in 30% H_2SO_4, thoroughly rinsed in low-Hg Milli-Q water, and sterilized if necessary. The exception was glass scintillation vials used for the assays, which were simply rinsed with low-Hg Milli-Q water. All culture transfers, media preparation, and assays were carried out in a clean lab (with HEPA-filtered air) and using a Class 100 laminar flow hood. The NaCl used in these experiments was muffled at 800 °C for 4 h to minimize the amount of organic materials present that could potentially bind Hg during the bioassay. None of the other chemical reagents could be muffled due to their low melting points.

Growth Media, Cell Preparation, and Harvesting. All assays, media preparation, and growth of cells were carried out under aerobic conditions in this work. Growth media contained 50 μg mL^{-1} kanamycin for selection and maintenance of plasmids. Inoculum was prepared in LB agar and broth (Difco). Prior to an assay, a single colony was inoculated into a test tube containing 5 mL of LB medium and grown for approximately 12 h. Fifty microliters of the culture containing *V. anguillarum* pRB28 or 100 μL of *V. anguillarum* pRB27 was transferred into a culture flask containing 5 mL of glucose minimal medium (GMM) prior to assays. GMM contained 16.7 mM glucose, phosphate buffer (40 mM K_2HPO_4, 20 mM NaH_2PO_4), 1 mM $MgSO_4$, 3 mM vitamin B_1, trace elements, and 20 mM NH_4Cl.

After 12−21 h (pRB28) or 21 h (pRB27) of incubation, an additional 20 mL of GMM was added to the flasks. The cells were then grown to mid-log phase and harvested. Depending

10.1021/es026366o CCC: $25.00
Published on Web 05/29/2003

TABLE 1. Effect of pH on Light Production by the Constitutive Strain *Vibrio anguillarum* pRB27, at Two Different NaCl Concentrations[a]

pH	H^+, mol/L	NaCl = 0 average cpm	NaCl = 1.7 mM average cpm	ratio to pH = 7.3, NaCl = 0: NaCl = 0	ratio to pH = 7.3, NaCl = 0: NaCl = 1.7 mM
7.30	5.01×10^{-8}	3.35×10^7	3.01×10^7	1.00	0.90
6.80	1.58×10^{-7}	3.08×10^7	3.16×10^7	0.92	0.94
6.56	2.57×10^{-7}	3.44×10^7	3.24×10^7	1.02	0.96
6.30	5.01×10^{-7}	2.30×10^7	2.37×10^7	0.68	0.71

[a] Assay reagents (glucose, ammonium sulfate, phosphate buffer, and mercury) were the same as used for the inducible strain, *Vibrio anguillarum* pRB28 in the experiments shown in Figures 1 and 2.

on the size of the inoculum and the length of the previous growth stage, *V. anguillarum* pRB28 required approximately 2.5−4 h to reach mid-log phase of growth and *V. anguillarum* pRB27 approximately 6−8 h. The slower growth of the pRB27 strain is likely due to the energy required for constitutive expression of the luminescence genes. All incubations were carried out at 26.5 °C with shaking at 100 rpm.

Cells were harvested by centrifugation at 10 000 rpm for 10 min at 4 °C. The supernatant was decanted, and the pellet was resuspended in 10 mL of buffer (6.7 mM P_i, pH 6.8) and centrifuged again. The pellet was then resuspended in 1−3 mL of buffer. The cell concentration was adjusted with P_i buffer to 10^6 cells/mL.

Assay Reagents. The concentrations of reagents in the assays for Hg(II) uptake included 5 mM glucose, 0.09 mM ammonium sulfate ($(NH_4)_2SO_4$), phosphate buffer (P_i), and either 0 or 1.7 mM NaCl. These reagents were added to Milli-Q water (for blanks), to $HgNO_3$ in Milli-Q water (standards), or to lake sample water and are necessary for the activity of the bacteria during the assay (*11*). The pH was manipulated in assays by altering the ratio of the mono and dibasic phosphate salts (NaH_2PO_4 or KH_2PO_4 and K_2HPO_4). The total phosphate concentration was held at 6.7 mM.

The *mer-lux* Bioassay. Assays were carried out in glass scintillation vials (assay volume = 20 mL). Defined solutions of Hg(II) with or without NaCl were prepared by the sequential addition of sterile Milli-Q water (18 mL) and sterile reagents to give final concentrations of 5 mM glucose, 0.09 mM $(NH_4)_2SO_4$), 6.7 mM inorganic phosphate (P_i), NaCl (0 or 1.7 mM), 0.4 mL cell suspension for a final cell concentration of 10^5 cells/mL (similar to cell numbers in natural lakes), and Hg(II) as $Hg(NO_3)_2$. For lake water samples, the Milli-Q was replaced by lake water, and all the same final concentrations of reagents and cells were added, except for NaCl. A maximum of 12−14 vials (standards + samples) was prepared for a single experiment, so that light production could be measured at 10 min intervals, with a scintillation counter (Beckman LS 6500) in noncoincidental mode. We examined the possibility that there might have been a change in dissolved Hg(II) concentration due to a change in adsorption onto the walls of the glass assay vials as the pH was adjusted. We did this by decanting the water from the assay vials after the pH was adjusted and measuring THg concentration in the decanted solutions. There was no difference with pH.

Hg Standard. The primary Hg standard was a 1 μg/mL $Hg(NO_3)_2$ solution prepared at Flett Research Ltd., using National Institute of Standards and Technology (NIST) standard reference material #3133, which was 10 g Hg/L in 10% HNO_3. Daily standards were prepared and used immediately, by diluting the primary standard (1 μg Hg mL^{-1}) twice in low-Hg (0.3−0.5 ng L^{-1}) Milli-Q water in Teflon vials for a final concentration of 62.5 ng Hg L^{-1}.

Analyses for Total Mercury Concentration. Reagents, water, and samples were analyzed for total mercury at Flett Research Ltd., Winnipeg, or at Frontier Geoscience, Seattle, using a cold vapor atomic flourescence spectrometer (Brooks Rand, Ltd. Model 2) and EPA Method 1631, as adapted from Bloom and Creselius (*12*). Samples were preserved for chemical analyses by adding 0.25 mL of concentrated hydrochloric acid per 125 mL sample.

Lake Water Collection. All sample containers (250 mL acid-washed Teflon bottles filled with Milli-Q and 1% clean HCl) were double-bagged and handled using clean technique, wearing Class 100 clean room vinyl gloves. Lake samples were taken about 10 a.m. and used in assays that afternoon.

Preparation of DOC Concentrate. A DOC concentrate was prepared by ultrafiltration, using surface water from L. 240 at the Experimental Lakes Area, February 7, 2001. The fraction between 10 K and 100 K was concentrated to 3200 μmol C/L and used in the bioreporter experiments on February 14, 2001. This concentrate included some Hg (0.015 ng Hg per μmol C), which was derived primarily from Hg present in the original lake water and also from contamination occurring during the concentration procedure. This Hg was not bioavailable (see Results, Table 2).

Calculation of Hg(II) Speciation. Hg(II) species in the inorganic ligand experiments were calculated using MINEQL+, version 4.5 (1998). Constants for Hg−PO_4 and Hg−HPO_4 complexes were added from Martell and Smith

TABLE 2. Effect of pH on Bioreporter Induction Slope in Presence and Absence of DOC[a]

pH	Hg added, ng L^{-1}	DOC, μmol C L^{-1}	induction slope (uncorrected), cpm min^{-1}	slope corrected to pH 7.3 light level,[b] cpm min^{-1}	slope minus blank, cpm min^{-1}	decrease due to DOC addition, %
7.30	0	0	0.005	0.005		
	2	0	0.103	0.103	0.10	
	0	85	0.011	0.011		
	2	85	0.040	0.040	0.03	71
6.84	0	0	0.008	0.009		
	2	0	0.135	0.154	0.14	
	0	85	0.008	0.009		
	2	85	0.066	0.072	0.06	57
6.53	0	0	0.007	0.008		
	2	0	0.127	0.153	0.14	
	0	85	0.010	0.012		
	2	85	0.099	0.124	0.11	23

[a] The concentration of Hg in the second column is Hg standard added; it does not include the approximately 1.3 ng Hg L^{-1} included in the DOC concentrate, most of which was unavailable. [b] The amount of light produced by *V. anguillarum* pRB27, the constitutive strain, was measured under all the same conditions as was Hg(II) uptake by *V. anguillarum* pRB28. Slopes were then corrected to take into account any differences in light production compared to light produced at pH 7.3, in Milli-Q water without DOC.

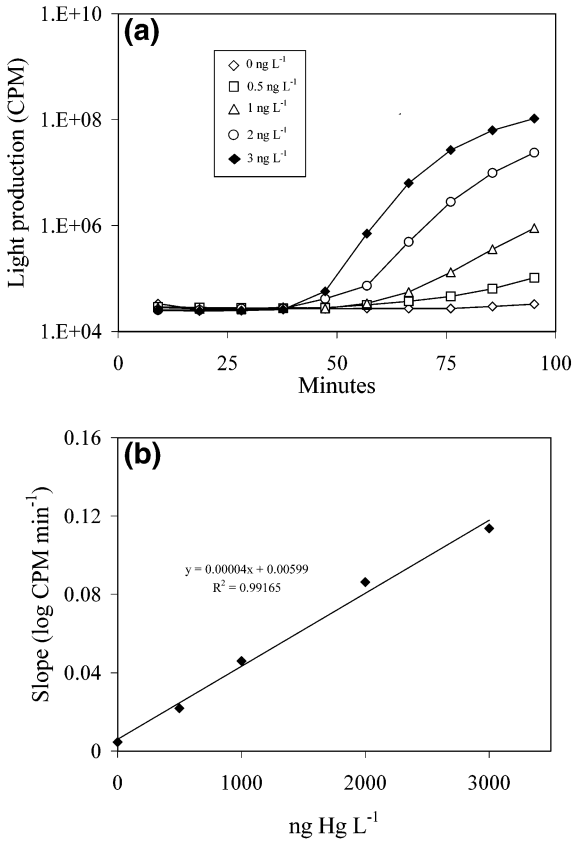

FIGURE 1. (a) Rate of light production (cpm/min) by the bioreporter (*V. anguillarum* pRB28) as a function of time after addition of 0, 0.5, 1, 2, and 3 ng Hg L^{-1}, added as $Hg(NO_3)_2$. The assay medium contained 5 mM glucose, 0.09 mM $(NH_4)_2SO_4$, and buffered with 6.7 mM inorganic phosphate at pH 7.3. (b) The initial log slope of the increase in light production as a function of total Hg concentration.

(*13*). Use of these introduced some uncertainty because only constants measured at ionic strength of 3 M are available, but constants for phosphate complexes with metals similar to Hg tend to be low in general (*13*), and these complexes were not very important in overall speciation.

Results

At a constant pH, when known amounts of Hg were added to the bioreporter cells, suspended in the assay medium, there was an initial lag period followed by a logarithmic increase in light output (Figure 1a). At a constant pH, the length of the lag period was shorter, and the rate of light increase was faster, at higher Hg(II) concentrations. This was the same as the response seen previously with *E. coli* cells containing the same *pRB28* plasmid (*14*) and for *V. anguillarum* pRB28 under aerobic conditions (*9*).

The response of the *V. anguillarum* pRB28 cells to Hg(II) was quantified by taking the maximum log-linear slope of light increase that occurred after Hg was added to cells in the assay medium (*14*). When the pH was held constant, this parameter increased linearly as Hg concentration increased (Figure 1b). In the many assays performed to date, r^2 values for this relationship have rarely been below 0.96 (see also ref *14*).

When mercury addition was held constant at 1 ng/L and the assays were done at four different pHs (7.3, 6.85, 6.5, and 6.3), there was a large increase in the bioreporter response as the pH was decreased (Figure 2). Over the range tested, the response was linearly related to the H^+ concentration (Figure 2). NaCl was added to some assays (at a final concentration of 1.7 mmol/L) in order to examine the effect

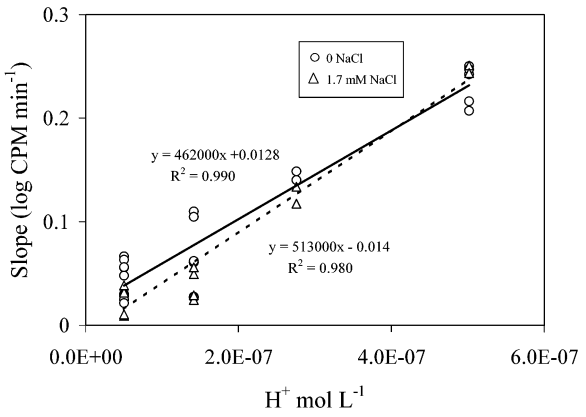

FIGURE 2. The initial log slope of the increase in light production as a function of hydrogen (H^+) concentration for experiments in which the pH was adjusted using $NaHPO_4$ and K_2HPO_4. Hg added was 1 ng L^{-1} and the assay medium contained 5 mM glucose, 0.09 mM $(NH_4)_2SO_4$, 6.7 mM inorganic phosphate, and either 0 (solid line) or 1.7 mmol L^{-1} NaCl (dashed line). Linear regressions were calculated for the means of assays done at each of four different H^+ concentrations.

of increasing the proportion of Hg present as the lipophilic species $HgCl_2$. However, there was no statistical difference ($P = 0.275$) between the slopes of the regression lines for the assays that were run with or without addition of NaCl (Figure 2), using a heterogeneity of slopes test.

The narrow pH range was chosen because increases in fish MeHg are seen in lakes during the early stages of acidification after only small changes in pH (*1*, *2*). Also, a larger range might have had effects on cell metabolism (specifically, on light production) that could interfere with interpretation of the data. We examined such possible effects on light production using a constitutive strain of *V. anguillarum* (*pRB27*) as described for the *E. coli* bioreporter in ref *14*. Light production was essentially the same at pHs 7.3, 6.8, and 6.56 but was about 30% lower at pH 6.3 at both concentrations of NaCl (Table 1). The induction slopes measured at pH 6.3 and shown in Figure 2 were adjusted upward by 30% to account for this metabolic decrease in light due to decreases in pH.

To ensure that the apparent pH effect was not an artifact caused by the changing ratio of Na:K in our assay media when adjusting pH by using mixtures of mono- and dibasic phosphate (NaH_2PO_4 and K_2HPO_4), we performed experiments at three different pHs using either our normal Na/K buffer mixture or an "all K" buffer that was made with KH_2PO_4 and K_2HPO_4. The bioreporter response was still dependent on pH (Figure 3), so we conclude that the effect on mercury uptake of our experiments was a pH effect and was not caused by differences in concentrations of Na and K ions.

While addition of 1.7 mM Cl^- did not affect Hg(II) uptake rate (Figure 2), addition of a small amount of a concentrate of lake water DOC markedly decreased the response of the bioreporter to Hg(II) (Table 2). For example, addition of only 85 μmol C/L as DOC (>10 K and <100 K) to 2 ng Hg/L decreased the response by 71% (Table 2) at pH 7.3. At lower pHs the decrease in bioreporter response due to the addition of DOC was less marked (Table 2). The DOC concentrate contained enough Hg associated with it to add 1.3 ng/L Hg to each assay vial, but the lack of response of the bioreporter to the DOC concentrate without further added Hg showed that the bioavailable fraction of this Hg was below the limit of detection of the bioreporter (~0.05 ng/L, Table 2). We expect that the native Hg associated with the DOC concentrate was bound tightly to sulfhydryl groups of the DOC molecules, which were too large to cross the cell membrane.

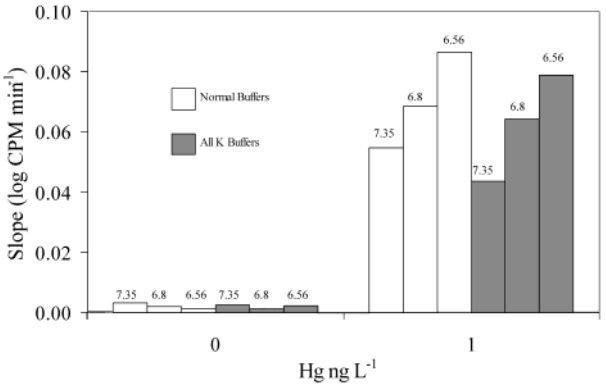

FIGURE 3. The initial log slope of the increase in light production in response to 1 ng Hg/L, as a function of hydrogen (H^+) concentration for experiments in which the pH was adjusted using either Na and K phosphate salts ($NaHPO_4$ and K_2HPO_4) or only K phosphate salts ($KHPO_4$ and K_2HPO_4), with a total inorganic phosphate concentration of 6.7 mM. Other assay components were 5 mM glucose and 0.09 mM $(NH_4)_2SO_4$.

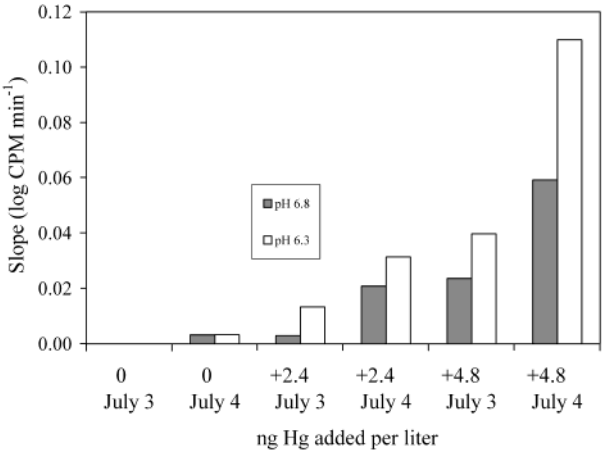

FIGURE 4. The effect of pH on bioreporter response in L. 658 water when 0, 2.4, and 4.8 ng Hg/L were added, on two dates. Assay reagents were also added (5 mM glucose, 0.09 mM $(NH_4)_2SO_4$, and 6.7 mM phosphate buffer, which determined the pH). Light production measured with the constitutive strain (*V. anguillarum* pRB27) was 93% lower at pH 6.3 than at pH 6.8, and so the slopes obtained at pH 6.3 were adjusted upward by dividing by 0.93.

When new $HgNO_3$ (2 ng Hg/L) was added, a percentage of this Hg became bound to these large molecules. However, as pH was lowered, either the new Hg became less bound, or the rate of non-DOC bound Hg uptake increased, or both.

We also experimented with whole lake water from Lake 658 at the Experimental Lakes Area, northwestern Ontario, which included DOC, particles, and a variety of inorganic cations and anions not found in our defined assay medium. Lake water was used as sampled, and with additions of 2.4 and 4.8 ng/L Hg(II) to make the measurement of bioreporter response more easily detectable. In lake water without added Hg, the lack of a detectable bioreporter response (detection limit = 0.1 ng Hg(II)/L) on July 3 was likely due to the low total Hg concentration (3.1 ng/L) and high DOC concentration (780 μmol C/L). This lake is currently undergoing experimental Hg additions as part of the Mercury Experiment To Assess Atmospheric Loading in Canada and the U.S. (METAALICUS (*15*)). There was an experimental addition of Hg to the whole lake after the July 3 samples were taken and before the July 4 sampling that increased the surface water concentration to 3.8 ng Hg/L. This small increase was enough to make the bioavailable Hg detetectable in unamended lake water on July 4 (Figure 4). When the pH of the lake water was lowered from the in situ pH of 6.8 and Hg was added, there were some differences in the degree of response, but in all cases there was an increase in Hg(II) uptake at the lower pH (6.3).

Discussion

Golding et al. (*9*) recently showed that uptake of Hg(II) by both *V. anguillarum* and by *E. coli* very likely occurs by a facilitated mechanism rather than by diffusion of neutrally charged Hg(II) species across the cell membrane, as was previously assumed (*8*). Data presented in this paper provide further evidence for facilitated Hg(II) uptake, using calculations of the speciation of Hg(II) at the various pHs and at the two different NaCl concentrations. Neutrally charged $HgCl_2$ was an important species in the assays where 1.7 mM NaCl was added, but it was insignificant in the assays where NaCl was not added (Figure 5a), yet rates of Hg(II) uptake at the two different NaCl concentrations were very similar at each pH (Figure 2). Neutrally charged $Hg(OH)_2$ was the other important species calculated to be in these assay solutions (Figure 5b), but it decreased in concentration as H^+ was increased. Thus, it did not appear that Hg(II) was entering the cells by diffusion of the relatively lipophilic and uncharged species $HgCl_2$ or $Hg(OH)_2$ across the cell membrane or that

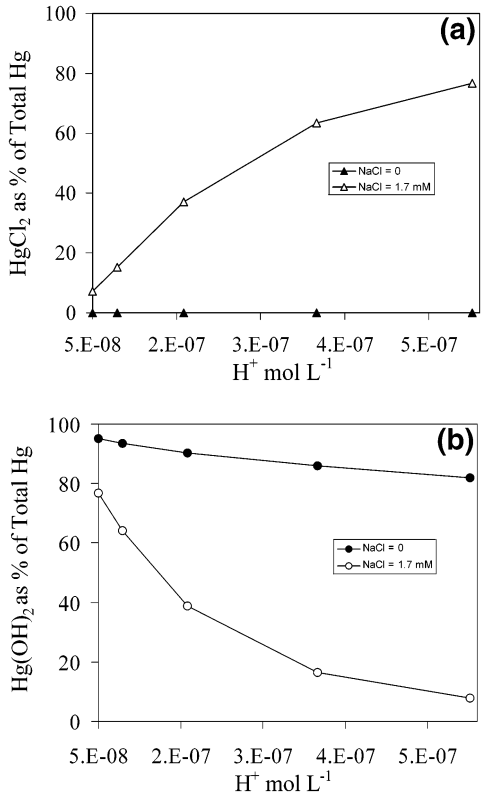

FIGURE 5. (a) Concentrations of $HgCl_2$ in the assay medium (5 mM glucose, 0.09 mM $(NH_4)_2SO_4$, and 6.7 mM phosphate buffer), when pH was varied from 7.3 ($H^+ = 1 \times 10^{-7}$ mol/L) to 6.3 ($H^+ = 5 \times 10^{-7}$ mol/L) and the NaCl concentration was either 0 or 1.7 mM, estimated using the speciation model MINEQL$^+$ version 4.5. (b) Concentrations of $Hg(OH)_2$ in the assay medium (5 mM glucose, 0.09 mM $(NH_4)_2SO_4$, and 6.7 mM phosphate buffer), when pH was varied from 7.3 ($H^+ = 1 \times 10^{-7}$ mol/L) to 6.3 ($H^+ = 5 \times 10^{-7}$ mol/L) and the NaCl concentration was either 0 or 1.7 mM, estimated using the speciation model MINEQL$^+$ version 4.5.

entry was controlled in any obvious way by bulk solution speciation. These experiments confirmed the previous work (*9*) demonstrating that Hg(II) uptake rate in these bacteria is controlled by the collective concentration of a number of available Hg(II) species, both charged and uncharged, which

is evidence that a cell-mediated process is important in determining how much Hg(II) enters the cell. The previous work also showed that the uptake process was affected by cell physiology, specifically, whether the cells were under aerobic or anaerobic conditions. We show in this report that the process is also affected by pH.

$HgCl_2$ and $Hg(OH)_2$ comprised 76−95% of all Hg(II) species in the different assays. Other Hg(II) species that were present in proportions greater than 1% were $HgHPO_4$, $HgPO4^-$, which increased with lower pH in the 0 NaCl assays but decreased in the 1.7 mM NaCl assays, and HgOHCl, which was only present when 1.7 mM NaCl was added and which increased and then decreased as pH was lowered. Thus, none of these species showed a consistent increase with lowered pH and so could not explain the increased Hg(II) uptake at lower pH.

Uptake of other metals by aquatic microorganisms is also affected by pH. However, for most metals a decrease in pH results in a decrease in the rate of metal uptake (e.g. ref *16*), and this is thought to occur because H^+ acts as a competitive inhibitor of uptake of positively charged metal ions. We know of no other metal uptake process that has been shown to be stimulated by H^+ except for bacterial Mn(II) uptake (*17*).

The lack of an increase of Hg(II) uptake by Cl^- addition (Figure 2) was similar to previous bioreporter experiments under aerobic conditions where addition of NH_3 or a variety of low molecular weight organic ligands (such as histidine) also did not increase or decrease Hg(II) uptake (*9*). The conclusion from these experiments was that the Hg(II) transport system could access Hg(II) complexed to a variety of small ligands that form neutral or positively charged complexes (*9*). In contrast to these low molecular weight ligands, addition of lake water DOC decreased uptake (Table 2), as expected due to tight binding of Hg to sulfhydryl groups and the large size of many of these molecules. The decrease was less, however, for the same concentration of Hg and DOC at lower pH (Table 2, Figure 4). Barkay et al. (*8*) also found an increase in bioavailable mercury concentration at reduced pH in samples with DOC added, similar to the results shown here. At the time of the Barkay et al. (*8*) publication, mercury researchers generally assumed that bacteria assimilate mercury by diffusion of neutrally charged species. Consequently it was concluded that the increase in Hg(II) uptake at lower pH was caused solely by desorption of Hg(II) from DOC or particles, which is expected because H^+ can displace Hg(II) by protonating sulfhydyl moieties that bind Hg (II) to DOC (*18*) or by replacing Hg(II) on negatively charged surfaces such as clay particles. While this is likely part of the explanation for increased Hg(II) uptake at lower pH in the presence of DOC (Table 2) and in lake water (Figure 4), we now know from the inorganic assays presented here (Figure 2) that another factor is likely an increase in the rate of the bacterial Hg(II) uptake process itself.

The ratio of Hg to DOC was obviously important in determining whether Hg(II) uptake was detectable by the bioreporter bacteria. Hg(II) uptake was easily detectable at pH 6.8 in the DOC addition experiments (Table 2), where the concentrations of Hg and DOC were 2 ng Hg/L and 85 μmol C/L, while uptake was barely detectable at the same pH in L. 658 water (Figure 4), with concentrations of 3 ng Hg/L and 780 μmol C/L, resulting in a lower ratio of Hg to DOC. In general, L. 658 surface water did not have detectable bioavailable Hg(II) during the summer of 2001, but streamwater flowing into the lake often did contain detectable bioavailable Hg(II) (unpublished data). Both total Hg and DOC concentrations were higher in the streamwater (10 ng Hg/L and 1500 μmol C/L) than in the lake, but the ratio of Hg to DOC was higher in the stream (meaning less of the total Hg was likely to be complexed by DOC), and the pH was lower (5.5). We think that both the Hg:DOC ratio and pH should be considered as important factors in predicting Hg(II) uptake by bacteria in aerobic surface waters.

A link between lake acidification and elevated concentrations of mercury in fish is well established (*1*, *2*). Recovery of lakes from acidification following controls on atmospheric emissions of SO_2 has been much slower than anticipated (*19*), and so lake acidification should continue to be considered as one of the contributing factors in the widespread incidences of elevated fish mercury concentrations in soft water lakes. In addition, it should be noted that pH is an important diel variable in surface waters, due to CO_2 uptake and respiration (e.g., ref *20*). There is also spatial variation of pH, especially at the sediment water interface (*21*), and in general pH is higher in marine waters than in freshwaters.

Hrabik and Watras (*22*) recently examined data for Little Rock Lake as it has recovered from acidification. They concluded that most of the Hg methylated in lakes is new Hg entering from the atmosphere and that a likely mechanism by which Hg concentration of fish decreased during deacidification was that there was a concomitant decrease in sulfate concentration and thus methylation by sulfate reducing bacteria. An additional factor could be that as pH increased, there would be less assimilation of Hg(II) into cells, leaving more Hg(II) available for photoreduction to Hg^0. This idea is supported by data showing that Hg^0 concentrations in lakes tend to be higher at higher pH (*23*; M. Amyot personal communcation). Also, if new Hg entering lakes is not assimilated by bacteria as quickly at higher pHs, there might be less delivery of particle associated bacteria (e.g., in fecal pellets) to the sediments, where methylation of new Hg occurs.

With respect to the microbial methylation process itself, it is known that higher methylation rates occur in lake water and surficial sediments when pH is lowered, independently of sulfate concentration (*4*), but the mechanism for this rate increase is not known. Bacterial cells commonly control their internal pH at near neutral values, and methylation is an internal process that would therefore be unaffected by external pH. Thus, the increased methylation at lower pH could be due to increased Hg(II) uptake by the methylating bacteria. Unlike passive uptake of neutral Hg species, which would be modeled in the same way for all bacterial species (e.g., ref *24*), facilitated metal uptake involves genetically determined cell constituents, and so the uptake process could differ from one species to another. While the bacterial species used in this study are not methylators, the observation of pH effects on methylation is a logical reason to examine methylating species for similar uptake processes.

In previous publications, links between pH and mercury transformations (e.g. methylation, Hg(II) reduction) have been interpreted as due to the effect of pH on DOC−Hg interactions or the covariance of pH and sulfate. In this study, we have shown that direct effects of pH on the bacterial Hg(II) uptake process itself also need to be considered in understanding mercury transformations in aquatic systems. The environmental relevance of this work is that the concept of mercury bioavailablility to bacteria needs to be changed. It should now include two components. The first is the concentration of bioavailable Hg species in the bulk solution. The second component is the bacterial Hg(II) uptake process itself, which can be affected by environmental factors such as pH or by changes in cell physiology and may differ from one species to another.

Acknowledgments

We are indebted to R. Sparling and G. Golding for their constructive comments and advice during the preparation of this paper and to T. Barkay and A. Summers for discussions of initial results. We also thank S. Kasian for statistical anal-

ysis and three anonymous reviewers for their constructive criticisms. This research was funded by the Electric Power Research Institute, ALCOA, and the Department of Fisheries and Oceans, Canada. This is Contribution No. 3 of the Mercury Experiment To Assess Atmospheric Loading in Canada and the United States (METAALICUS).

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Received for review November 26, 2002. Revised manuscript received March 21, 2003. Accepted March 31, 2003.

ES026366O