Microbial δ-aminolevulinate dehydratase as a biosensor of lead bioavailability in contaminated environments

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Abstract

Delta-aminolevulinate dehydratase (ALAD) is a phylogenetically conserved enzyme that is responsible for the synthesis of porphobilinogen in a key step involving the production of heme. ALAD is a metalloprotein that requires magnesium or zinc for its activity, depending on the species. In humans, ALAD activity is zinc-dependent, and has been used extensively as a biomarker for lead (Pb) exposure and toxicity. ALAD activity in other multicellular eukaryotes has been used in ecotoxicology as a biomarker for environmental lead pollution. Because microorganisms are sensitive indicators of toxicity at the fundamental level of ecological organization, we hypothesized that bacterial ALAD can serve as a reliable biomarker for lead bioavailability in contaminated environments. In this study, ALAD activity in an environmental strain of Pseudomonas putida was investigated to evaluate potential inhibition by Pb and other toxic metals. There was a statistically significant dose–response relationship between ALAD activity in cells of P. putida, ATCC 700097 and [Pb] (Pearson correlation coefficient \( r^2 = 0.97 \), and \( P < 0.001 \)). The highest level of inhibition of ALAD activity was approximately 74% of the normal level when cells were incubated with \([Pb^{2+}] > 500 \) μM. The relationship between Pb and ALAD activity was statistically described by \(\log(Pb) = 3.68 - 1.41[ALAD \text{ Activity}]\). In protein extracts of P. putida, ALAD activity was reduced by up to 85% in response to 500 μM of Pb. A higher concentration of Pb was needed to produce a comparable level of ALAD inhibition in P. putida cells seeded into natural freshwater, suggesting that Pb was not completely bioavailable in the water samples. In contrast to the findings with P. putida, the ALAD activity in a known metal-resistant P. aeruginosa PU21 (Rip64) was not sensitive to Pb exposure. Therefore, the sensitivity of ALAD to Pb in complex heterogeneous ecosystems depends on the molecular diversity of ALAD in predominant species, and on the bioavailability of Pb.

Keywords: Enzyme; Biosensor; Lead; Delta-aminolevulinate dehydratase

1. Introduction

The global distribution of lead (Pb) coupled with its well-documented deleterious effects on biological systems make Pb one of the most hazardous environmental toxicants. The background concentration of Pb in the Earth’s crust is 16 μg/g, but human industrial activities have resulted in Pb concentrations several orders of magnitude above background levels in soils (up to 5000 μg/g), freshwater (up to 10 μg/l), and air (up to 10 μg/m³) (Pang, 1995). Ecotoxicological studies on Pb have traditionally focused on elucidating the interactions among exposure pathways, internal doses, and health effects (Pang, 1995). Although questions related to the bioavailability of Pb and its biotransformation from inorganic to organic forms have long been recognized as important, there is a paucity of information on how these factors affect the overall distribution and potency of Pb in ecosystems (Wong et al., 1975).

Previous studies have shown that microbial biofilms play important roles in controlling toxic metal concentrations in natural ecosystems. For example, Pseudomonas atlanticus has been implicated in the formation of biofilms where trace metal distributions are affected by the organisms’ ability to modify adsorption properties of inorganic surfaces (Fabiano et al., 1994; Hsieh et al., 1994; Fernandez-Leborans et al., 1998). One of the consequences of biofilm interaction with toxic metals is bioaccumulation, which provides opportunities for monitoring bioavailability and ecophysiological effects. In this respect, the activity of δ-aminolevulinic acid dehydratase (ALAD) has been used extensively as a biomarker for lead exposure in humans and some aquatic eukaryotes (Conner and Fowler, 1994; Overman and Kraijcek, 1995; Claudio et al., 1997; Morita et al., 1997; Bishop et al., 1998; Burden et al., 1998; Fleming et al., 1998; Xie et al., 1998). ALAD is a 128-kD metalloprotein that plays a
role in heme synthesis (Tanaka et al., 1995). Few bacterial ALAD systems have been described, and the metallic component of ALAD differs among species (Chauhan and O’Brian, 1995; Mamet et al., 1996). For example, zinc is required for ALAD activity in humans, yeasts and *Escherichia coli*, whereas magnesium is required in *Bradyrhizobium japonicum* (Chauhan and O’Brian, 1995). Moreover, the sensitivity of ALAD to toxic metals depends on the identity of the metallic co-factor (Chauhan and O’Brian, 1995; Tanaka et al., 1995; Ogunseitan et al., 1999). A genetic polymorphism at the ALAD locus has been described in humans, with implications for susceptibility to lead poisoning (Wetmur, 1994). The evolutionary origin of the ALAD polymorphism has not been established, but it is clear that geographic and strain-specific factors define the distribution of the two recognized ALAD alleles (Fleming et al., 1998). It has also been shown that organisms bred in environments containing high levels of Pb are endowed with multiple copies of the ALAD gene, suggesting the contribution of a strong environmental selective pressure to the evolution of ALAD genotypes (Bishop et al., 1998).

In many ecological systems, bacterial populations are sensitive indicators of bioavailability and physiological consequences of toxic compounds, particularly in situations where anthropogenic and geochemical factors contribute to dynamic shifts in chemical speciation and concentration (Ogunseitan, 1999). It is for this reason that we embarked on the project to characterize the potential for using the inhibition of bacterial ALAD as a biosensor for Pb pollution. The results show that the ALAD activity in *P. putida* is sensitive to Pb, and the dose–response of ALAD sensitivity is a function of Pb bioavailability at both the cellular and the molecular levels of physiological function.

2. Materials and methods

2.1. Bacterial strains and culture conditions

*Pseudomonas putida* ATCC 70097 was originally isolated from an urban wastewater stream (Ogunseitan, 1996). The strain has no known metal resistance phenotype, but it has the capacity to metabolize certain aromatic organic compounds of environmental importance. *P. aeruginosa* PU21 (liv leu Str Rif) carrying the 142.5 kb plasmid Rip64 is a derivative of strain PAO1. *P. aeruginosa* PU21 has a mercury resistance phenotype encoded by a mer operon located on the plasmid (Ogunseitan, 1998). Bacteria were routinely cultivated on Tryptic Soy Broth (TSB; Difco, Michigan). When desired, 12% agar was added to solidify the medium. Because TSB contains materials that can interfere with bacterial interaction with metals, a special Heavy Metals Medium (HMM) was used in experiments involving bacterial exposure to metals. The composition of HMM is as follows: 40 mM of MOPS buffer, pH 7.2; 50 mM of KCl; 10 mM of NH₄Cl; 0.5 mM of MgSO₄; 1 mM of glycerol-2-phosphate; 1 µM of FeCl₃; 0.4% of glucose. To prepare HMM, a solution of MOPS buffer, KCl, and NH₄Cl was filter-sterilized and added to autoclaved glucose solution, and filter-sterilized glycerol-2-phosphate and FeCl₃ solutions. Inorganic metal solutions (lead nitrate, magnesium sulfate, manganese sulfate, cobalt chloride, nickel nitrate, or zinc sulfate) were also filter-sterilized before addition to HMM. All chemicals used in this study were of pure analytical grade quality purchased from Sigma (St. Louis, MO).

2.2. Freshwater samples

Natural freshwater was collected from the Upper Newport Bay Ecological Reserve, an urban watershed that drains a 254 km² region in southern California. The inflow of water into the bay is primarily through San Diego Creek and storm water drains from an intricate network of roads. Water samples were collected as previously described (Ogunseitan, 1999), transferred to the laboratory, and used within an hour of collection. The bacterial population density of the freshwater was determined by serial dilution and spread plating on Standard Methods Agar (Difco; Becton-Dickinson, Sparks, MD).

2.3. Effects of various metals on ALAD activity

The *P. putida* and *P. aeruginosa* strains were cultivated in TSB until the cell population density reached 10⁸ colony forming units (CFU) per milliliter. The cells were harvested by centrifugation, washed and re-suspended in HMM. Protein was extracted from the cultures as previously described (Ogunseitan, 1998, 1999), and summarized below. One hundred micrograms aliquots of protein from each bacterial strain were incubated for 1 h at room temperature in 100-µl solutions containing 500 µM of Pb²⁺, Zn²⁺, Ni²⁺, Co²⁺, Mn²⁺, or Mg²⁺. After the incubation period, the specific activity of ALAD in the protein extracts was determined as described below. Control experiments without metals were conducted for each bacterial strain. All experiments were performed in triplicate.

2.4. Dose–response effect of Pb on ALAD activity

The two bacterial strains *P. putida* and *P. aeruginosa* were cultivated to high cell density (10⁸ CFU/ml) in TSB. The cells were harvested by centrifugation, washed and re-suspended in HMM. Protein was extracted from triplicate cultures as described below, and 100-µg aliquots of protein were incubated for 1 h at room temperature with Pb²⁺ at concentrations of 0, 100, 200, or 500 µM. After incubation, ALAD activity was determined as described below.

In addition to the experiments where only extracted protein was exposed to Pb²⁺, a separate set of triplicate experiments was conducted where intact cells were exposed to Pb²⁺ for 1 h at 25°C prior to protein extraction and the determination of ALAD activity. The second set of experiments was designed to test whether bacterial uptake limited
Fig. 1. The catalytic reaction of ALAD. Two molecules of ALA engage in a Knorr-type condensation reaction to produce porphobilinogen, the pyrrole building block of hemes, chlorophylls, and corrins.

5-Aminolevulinic Acid (ALA)  Porphobilinogen (PBG)

Fig. 2. Spectrophotometric curve associated with ALAD activity in protein extracts of *P. putida* ATCC 700097 (triangles), showing maximum absorption at 553 nm. Specificity of the reaction is shown by the absence of an absorption peak in the absence of the substrate, ALA, in the reaction mixture (circles). ALAD inhibition by Pb reduces the size of the absorption peak, but does not change its location (squares).
Table 1
Effect of various metal ions on ALAD activity in P. putida ATCC 700097

<table>
<thead>
<tr>
<th>Metal</th>
<th>Specific activity of ALAD (µmol of porphobilinogen mg⁻¹ min⁻¹)</th>
<th>% Inhibition (−) or enhancement (+) of ALAD activity by metals¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co²⁺</td>
<td>0.116 (0.003)</td>
<td>38 (+)</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.111 (0.013)</td>
<td>32 (+)</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>0.086 (0.009)</td>
<td>2 (+)</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>0.125 (0.010)</td>
<td>49 (+)</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>0.020 (0.002)</td>
<td>76 (−)</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>0.074 (0.008)</td>
<td>12 (−)</td>
</tr>
<tr>
<td>No metal</td>
<td>0.084 (0.007)</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ 100 µg of extracted protein was exposed to 500 µM of metal salts.
² The values are based on three experimental replicates, and the numbers in parentheses represent standard deviation.
³ The effects on ALAD are statistically significant for Pb²⁺ (P = 0.007); but not for Co²⁺ (P = 0.994), Mg²⁺ (P = 0.199), Mn²⁺ (P = 0.904), Ni²⁺ (P = 0.053), or Zn²⁺ (P = 0.368).

were centrifuged at 3000 × g for 10 min at 4°C in order to collect pelleted cells. The supernatant was discarded and the cell pellet was resuspended in 2 ml of a cold lysis buffer solution containing 20 mM of Tris–Cl, 1 mM of DTT, and 1 mM of phenylmethylsulfonl fluoride, pH 7.4. The resuspended cells were lysed by pulse-sonication using a 3-mm microtip (Fisher Scientific Sonic Dismembrator 550; Tustin, CA). The sonicator was equipped with an ice-bath to prevent protein denaturation during the lysis process. Lysed cell suspensions were centrifuged at 25,000 × g for 25 min at 4°C and the protein supernatant was transferred into a fresh tube. The concentration of proteins in the extract was determined immediately by means of the Bradford Assay (USB Biochemicals, Cleveland, OH). Enzyme assays were performed on fresh extracts, and excess protein was stored at −20°C.

2.7. Determination of ALAD activity

One hundred micrograms of each protein extract was added into a fresh 1.5-ml microcentrifuge tube and the volume was adjusted to 200 µl with 50 mM potassium phosphate buffer (pH 7.2) containing 2 mM of dithiothreitol (DTT). Another 200 µl of 50 mM potassium phosphate buffer (pH 7.2) containing 6 mM of β-aminolevulinate and 1 mM of DTT was added, and the reaction was initiated by incubation at 42°C for 2 h. To stop the reaction, 1 ml of 4% trichloroacetic acid was added to each tube. The reactions were centrifuged for 2 min at 12,000 × g and 100 µl of each supernatant was transferred to a microtiter well containing 100 µl of modified Ehrlich’s reagent (3.4 ml of acetic acid, 1.6 ml of 70% perchloric acid, 1 ml of 0.25 M mercury chloride, and 0.1 g of p-dimethylaminobenzaldehyde (DAB)). The development of a pink color, which represents porphobilinogen, the product of ALAD catalysis (Fig. 1), was monitored by using a scanning spectrophotometer (Spectramax 250; Molecular Devices, Sunnyvale, CA). The maximum absorption for porphobilinogen was found to be 553 nm (Fig. 2), and absorption at this wavelength was used to quantify subsequent assays. ALAD activity was expressed as micromole porphobilinogen produced per milligram protein per minute, where the molar absorption coefficient of porphobilinogen equals 6.8 × 10⁴ M⁻¹ cm⁻¹ measured under the same conditions used in the present experiments (Battistuzi et al., 1981).

2.8. Statistical analysis

The SPSS statistics software program (Microsoft, Seattle, WA) was used for statistical analysis of all data, including paired-sample t-tests, correlations, regressions, analysis of variance, and significance testing.
Table 3

Effect of Pb on ALAD activity in protein extracts from P. putida ATCC 900097

<table>
<thead>
<tr>
<th>Pb concentration (µM) a</th>
<th>Specific activity of ALAD (µmol of porphobilinogen mg⁻¹ min⁻¹) b</th>
<th>% Inhibition of ALAD activity by [Pb] c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.136 (0.0038)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.036 (0.0053)</td>
<td>74</td>
</tr>
<tr>
<td>200</td>
<td>0.025 (0.0025)</td>
<td>82</td>
</tr>
<tr>
<td>500</td>
<td>0.020 (0.0015)</td>
<td>85</td>
</tr>
</tbody>
</table>

a Pb was added to 100 µg of protein extracted from cells grown in the absence of Pb.
b The values are based on three experimental replicates, and the numbers in parentheses refer to standard deviation.
c The statistical model derived from the experimental data for predicting [Pb] is log[Pb] = 2.93 – 21.7[ALAD Activity]; r² = 0.984; P = 0.008.

3. Results

3.1. Specificity of the effect of Pb on bacterial ALAD activity

The results of experiments conducted to test the effect of various metals on ALAD activity in protein extracts from P. putida and P. aeruginosa are presented in Tables 1 and 2. For P. putida, only exposure to Pb produced a statistically significant (P = 0.007) inhibition of ALAD activity when compared to the control samples (Table 1). The data show that exposure of protein extracts from P. putida to 500 µM of Pb produced a 76% inhibition of ALAD activity. Zinc also appeared to exert an inhibitory effect on ALAD activity, but this effect was not statistically significant (P = 0.368). Cobalt, magnesium, manganese, and nickel appeared to enhance ALAD activity, although these effects did not produce statistically significant differences from the control. Of the six metals tested, Pb gave the most pronounced effect on ALAD activity in P. putida, and its effect was inhibitory (Table 1).

In contrast to the observations with P. putida, none of the metals tested produced a remarkable effect on the ALAD activity of protein extracts from P. aeruginosa (Table 2).

Table 4

Effect of Pb on ALAD activity in protein extracts from P. aeruginosa PU21

<table>
<thead>
<tr>
<th>Pb concentration (µM) a</th>
<th>Specific activity of ALAD (µmol of porphobilinogen mg⁻¹ min⁻¹) b</th>
<th>% Inhibition of ALAD activity by [Pb] c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.166 (0.0021)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.165 (0.0021)</td>
<td>0.6</td>
</tr>
<tr>
<td>200</td>
<td>0.160 (0.0010)</td>
<td>4</td>
</tr>
<tr>
<td>500</td>
<td>0.165 (0.0031)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a Pb was added to protein extract from cultures grown without Pb.
b The values are based on three experimental replicates, and the numbers in parentheses refer to standard deviation.
c The change in ALAD activity in response to [Pb] is not statistically significant: r² = 0.213; P = 0.538.

3.2. Dose–response effect of Pb on ALAD activity

The results of experiments conducted to determine whether there is a dose–response effect of Pb on ALAD activity in the two strains are presented in Tables 3–6. The effects of 0–500 µM of Pb on ALAD activity in protein extracts from P. putida is reported in Table 3. There was a statistically significant dose–response relationship described by the equation:

log[Pb] = 2.93 – 21.7[ALAD Activity].

The Pearson correlation coefficient was −0.992 (r² = 0.984), and P = 0.008. The strongest ALAD inhibition measured was 85% when protein extracts were incubated with 500 µM of Pb (Table 3).

In contrast to P. putida, there was no statistically significant change in ALAD activity in protein extracts from P. aeruginosa as a result of Pb doses (Table 4). The comparable Pearson correlation coefficient for the interaction between ALAD of P. aeruginosa and Pb was −0.462 (r² = 0.213; P = 0.538).

Table 5 shows the data from experiments conducted to test the exposure of intact cells of P. putida to various concentrations of Pb. There was a statistically significant inverse dose–response relationship between Pb concentration and ALAD activity. The concentration of Pb can be predicted by the ALAD activity in exposed intact cells as follows:

log[Pb] = 3.68 – 1.41[ALAD Activity].

The Pearson correlation coefficient of the relationship was −0.985 (r² = 0.971) and P < 0.001. The equation is accurate for [Pb] < 500 µM.

In contrast to the findings with P. putida, there was no statistically significant change in the relationship between Pb concentration and ALAD activity in exposed P. aeruginosa cells (Table 6). The ALAD activity in this strain...
The bacterial population density in the freshwater sample was 

\( (3.4 \pm 1.2) \times 10^4 \) CFU ml\(^{-1}\). The yield of protein in uninoculated freshwater samples was \( (0.06 \times 0.002) \) mg ml\(^{-1}\). The ALAD activity in uninoculated freshwater was sensitive to Pb (36% inhibition by 1000 \( \mu \)M of Pb), but at a lower level than the specific activity of ALAD in freshwater microcosms inoculated with \( P. putida \) where the inhibition was 60–67% (Table 7). The ALAD activity in inoculated freshwater did not change with increasing cell density of the \( P. putida \) inoculum. Similarly, the statistically significant \( (P < 0.001) \) level of inhibition of ALAD activity by Pb in these microcosms was approximately the same (Table 7).

### 3.3. Effect of Pb on ALAD activity in bacteria seeded into natural freshwater

The bacterial population density in the freshwater sample was 

\( (3.4 \pm 1.2) \times 10^4 \) CFU ml\(^{-1}\). The yield of protein in uninoculated freshwater samples was \( (0.06 \times 0.002) \) mg ml\(^{-1}\). The ALAD activity in uninoculated freshwater was sensitive to Pb (36% inhibition by 1000 \( \mu \)M of Pb), but at a lower level than the specific activity of ALAD in freshwater microcosms inoculated with \( P. putida \) where the inhibition was 60–67% (Table 7). The ALAD activity in inoculated freshwater did not change with increasing cell density of the \( P. putida \) inoculum. Similarly, the statistically significant \( (P < 0.001) \) level of inhibition of ALAD activity by Pb in these microcosms was approximately the same (Table 7).

### 4. Discussion

The aim of this study was to explore the possibility of

using bacterial ALAD activity as a sensor for biologically available Pb that poses considerable hazard to the ecosystem and human health in contaminated environments (Pang, 1995). The need for a biologically responsive sensor for toxic chemicals in the environment is based on several observations on the nature of limiting factors influencing toxicant bioavailability in the environment (Newman and Jagoe, 1994). A metal is considered to be in bioavailable form when it is taken up by organisms and is subsequently accessible to the physiological processes upon which it exerts an effect. Several factors, including physical (e.g. physical adsorption, occlusion in solid phase), chemical (e.g. ligand complexation), and biological (e.g. physiological adaptation and particular species characteristics) parameters can influence Pb bioavailability. The small size of bacteria, their ubiquitous distribution in the environment, and their metabolic versatility make them prime targets for research in environmental biosensors. This study extends previous investigations of the effect of Pb on ALAD to include the sensitivity of this enzyme in bacterial populations and communities.

There are three salient findings in this study. The first is that the ALAD activity in an environmental bacterial isolate, \( P. putida \) ATCC 700097 can be used as a biosensor for Pb (Tables 1, 3, 5, and 7). Out of the six metals tested, only Pb exerted a statistically significant inhibitory effect on ALAD activity in this strain. However, the results also show that other metals, in particular Ni appear to enhance the activity of ALAD in \( P. putida \), although these effects are not statistically significant. This observation is nevertheless interesting with respect to previous findings suggesting that the ALAD of \( Bradyrhizobium japonicum \) can be genetically engineered to vary metal sensitivity, although Pb was not included in that study (Chauhan and O‘Brian, 1995). The co-occurrence of metal ions in contaminated environments may lead to a situation where certain metals will compete for active sites on physiological targets, and the ultimate effect at the population level will depend on the summation of various influences. The present study did not include the interactive effect of various metals, and the pertinent experiment involving various metals was conducted with protein extracts, as opposed to intact cells (Tables 1 and 2).

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**Table 6**

Effect of Pb on ALAD activity in \( P. aeruginosa \) PU21Cells

<table>
<thead>
<tr>
<th>Pb concentration (( \mu )M)(^a)</th>
<th>Specific activity of ALAD (( \mu )mol of porphobilinogen mg(^{-1}) min(^{-1}))(^b)</th>
<th>% Change in ALAD activity due to [Pb](^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.20 (0.002)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.21 (0.0015)</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>0.22 (0.0015)</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>0.22 (0.001)</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>0.23 (0.0015)</td>
<td>15</td>
</tr>
<tr>
<td>500</td>
<td>0.22 (0.0015)</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>0.23 (0.003)</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^a\) Pb was added to \( 10^3 \) cells prior to protein extraction.

\(^b\) The values are based on three experimental replicates. Numbers in parentheses refer to standard deviation.

\(^f\) The changes in ALAD activity produced by Pb are statistically significant \( (P < 0.001) \).

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**Table 7**

Effect of Pb on ALAD activity in \( P. putida \) ATCC 700097 cells seeded into freshwater

<table>
<thead>
<tr>
<th>( P. putida ) cell density per milliliter of San Diego Creek Water(^a)</th>
<th>Specific activity of ALAD(^d) (( \mu )mol porphobilinogen mg(^{-1}) protein min(^{-1})) in protein extracted from microcosm contents</th>
<th>% Inhibition of ALAD activity by [Pb](^j) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without Pb</td>
<td>With Pb</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.51 (0.002)</td>
<td>0.32 (0.002)</td>
</tr>
<tr>
<td>( 10^2 )</td>
<td>2.21 (0.005)</td>
<td>0.77 (0.003)</td>
</tr>
<tr>
<td>( 10^4 )</td>
<td>2.32 (0.016)</td>
<td>0.76 (0.007)</td>
</tr>
<tr>
<td>( 10^5 )</td>
<td>2.28 (0.012)</td>
<td>0.91 (0.032)</td>
</tr>
</tbody>
</table>

\(^a\) Lead nitrate was added to one set of microcosms containing 100 ml San Diego Creek water to a final concentration of 1000 \( \mu \)M.

\(^d\) Mean of three replicate experiments. Numbers in parentheses refer to standard deviation.

\(^j\) The changes in ALAD activity produced by Pb are statistically significant \( (P < 0.001) \).
Therefore, the effect of Pb as an inhibitor of ALAD may potentially override the effect of other metals on the basis of specificity and bioavailability, because intact cells exposed to Pb also show intense dose–response inhibition of ALAD activity (Table 5). It is preferable that a sensor of bioavailability uses intact cells as opposed to free or immobilized enzymes because uptake factors and membrane transport can seriously affect biological availability of metals (Newman and Jagoe, 1994). Therefore, the data reported in Table 5 are more pertinent to a predictive model of Pb biosensor. The ALAD activity in cells of P. putida is sensitive to as little as 10 μM of Pb in growth medium, but beyond 500 μM of Pb, the dose–response effect was saturated (Table 5). The range of [Pb] to which ALAD in P. putida cells is sensitive is realistic in contaminated environments (Pang, 1995).

The second salient finding in this study is that the ALAD activity in certain bacteria is not sensitive to Pb. In this study, the finding that the ALAD activity both in intact cells and protein extract from P. aeruginosa PU21 is not sensitive to Pb provided two valuable pieces of information. The observations with P. aeruginosa served a critical control purpose to support the findings with P. putida. Additionally, the lack of sensitivity of ALAD activity in P. aeruginosa to Pb suggests that there exists molecular mechanisms by which bacteria have adapted to Pb toxicity. The most likely mechanism by which the ALAD of P. aeruginosa is resistant to Pb is that there is a modification in the amino acid sequence of the polypeptide chains such that the metallic component required for enzyme activity cannot be easily replaced by Pb. This explanation is based on the reported observation of the occurrence of two ALAD isozymes, both requiring Zn for activity, but with different Pb-binding affinities (Wetmur, 1994). It is also possible that the metal component required for ALAD activity is different in the two bacterial species, a condition that has been shown to alter the metal sensitivity of ALAD (Chauhan and O’Brian, 1995). The P. aeruginosa strain used in this study exhibits mercury resistance through the synthesis of mercuric reductase (Ogunseitan, 1998), but there is no evidence in the literature that mercuric reductase interacts with Pb (Cummings and Walsh, 1992).

The third salient result of the present study is observation that the concentration of Pb needed to produce comparable levels of ALAD inhibition was different in natural freshwater than in HMM (Table 7). When P. putida cells were inoculated into freshwater amended with 1000 μM of Pb, a maximum of 67% inhibition of ALAD activity was observed (Table 7). This level of inhibition is comparable to that produced by 100–500 μM of Pb in HMM. Reduced bioavailability of Pb in freshwater is offered as an explanation for this discrepancy. Ligands, which are anions or molecules that form coordination compounds or complexes with metals, commonly occur in natural waters (Newman and Jagoe, 1994), and may be responsible for the reduced bioavailability of Pb in San Diego Creek water. The most important inorganic ligands that form complexes with metals in natural waters are F–, Cl–, SO42–, OH–, HCO3–, CO32–, HPO42–, and NH3, with HS–, and S2– being influential in anoxic waters (Newman and Jagoe, 1994). The covalent bonding tendency (Δβ) and the ionic bonding tendency (Z2/r) of metals are influential in determining the types of ligands that could reduce bioavailability (Newman and Jagoe, 1994). Metals such as Pb are classified as borderline (for Pb, 2 > Δβ > 0; and 2.5 < Z2/r < 7). As such, not all of these ligands are relevant to the bioavailability of Pb, but clearly the presence of inorganic phosphate ions in freshwater, which is avoided in HMM, may influence Pb bioavailability. As discussed above, several complex factors affect the availability of toxic metals in the environment, and many of these factors are poorly understood. The proposed Pb biosensor based on inhibition of ALAD activity described in this study could aid investigations of physical and chemical factors that influence the bioavailability of Pb in environmental samples.

Further studies to improve the microbial biosensor approach to Pb contamination include the characterization of the molecular diversity of ALAD in natural microbial communities, for the purpose of developing a sensitive in situ assay for ALAD activity. Studies on the immobilization of ALAD for a rapid assay for total biologically active Pb in contaminated environments are also warranted.

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