The Molecular Mechanism of Lead Inhibition of Human Porphobilinogen Synthase

Human porphobilinogen synthase (PBGS) is a main target in lead poisoning. Human PBGS purifies with eight Zn(II) per homo-octamer; four ZnA have predominantly nonsulfur ligands, and four ZnB have predominantly sulfur ligands. Only four Zn(II) are required for activity. To better elucidate the roles of Zn(II) and Pb(II), we produced human PBGS mutants that are designed to lack either the ZnA or ZnB sites. These proteins, MinusZnA (H131A, C223A) and MinusZnB (C122A, C124A, C132A), each become purified with four Zn(II) per octamer, thus confirming an asymmetry in the human PBGS structure. MinusZnA is fully active, whereas MinusZnB is far less active, verifying an important catalytic role for ZnB and the removed cysteine residues. Kinetic properties of the mutants and wild type proteins are described. Comparison of Pb(II) inhibition of the mutants shows that ligands to both ZnA and ZnB interact with Pb(II). The ZnB ligands preferentially interact with Pb(II). At least one ZnA ligand is responsible for the slow tight binding behavior of Pb(II). The data support a novel model where a high affinity lead site is a hybrid of the ZnA and ZnB sites. We propose that the lone electron pair of Pb(II) precludes Pb(II) to function in PBGS catalysis.

Porphobilinogen synthase (PBGS), EC 4.2.1.24, also known as 5-aminolevulinic acid dehydratase, an enzyme that functions in the first common step in tetrapyrrole biosynthesis (e.g. heme and chlorophyll), is a highly conserved protein throughout evolution but has significant phylogenetic variation in the number and types of metal ions that function in catalysis or at allosteric sites (1). The human PBGS protein and its metal binding properties are of particular interest, because human PBGS is a primary target for the environmental toxin lead (2). Past studies using yeast PBGS as a model for human lead poisoning are limited because of a phylogenetic difference in the four Zn(II) that are essential for the activity of mammalian PBGS (3, 4). There are two codominant alleles, ALAD1 and ALAD2, which encode the human PBGS variants at position 59, K59 and N59, respectively. Recent data on the isolated K59 and N59 proteins do not support the epidemiological data that has correlated these alleles with a differential susceptibility to lead poisoning (3, 5).

All PBGS proteins appear to be homo-octamers, and there is evidence for and against half-sites reactivity, including cases where any one type of metal is bound at four per octamer (e.g. Refs. 6–10). Mammalian PBGS enzymes purify with Zn(II) bound at a stoichiometry of eight per octamer (6, 7, 11). Only four Zn(II) appear to be tightly bound and only four Zn(II) appear to be required for full catalytic activity of human PBGS (3), consistent with data on bovine PBGS (6, 7, 11, 12). Preliminary data obtained on human PBGS show that Pb(II) can displace about half the enzyme-bound Zn(II) (3). There are two outstanding questions in the structure and mechanism of human PBGS: 1) Which of the two types of Zn(II) corresponds to the four Zn(II) that are essential for the activity of mammalian PBGS? and 2) What extent do the two Zn(II) sites play in the Pb(II) inhibition of enzyme activity?

The crystal structure of yeast PBGS (Protein Data Bank code 1w56) revealed Zn(II) at two different sites with ligands that are consistent with prior chemical modification data (12, 13). In the crystal structure, the two Zn(II) sites were not fully occupied (14). The most highly populated Zn(II) site shows the metal bound to a cluster of cysteine residues, which in the human protein correspond to Cys-122, Cys-124, and Cys-132. The Zn(II) that binds to this cysteine-rich site is defined as ZnB (8). Although ZnB is 8 Å from an active site lysine, its coordination environment more closely resembles structural rather than catalytic Zn(II). A lesser-populated Zn(II) site of yeast PBGS was only seen in a difference map and shows Zn(II) with an incomplete coordination shell consisting of one histidine and one cysteine, which in the human protein correspond to His-131 and Cys-223. The Zn(II) that binds to this site is known as ZnA (8). Although these ligands are typical of catalytic Zn(II), the low occupancy and 12 Å distance from a Schiff base-forming lysine indicate ZnA to be of questionable significance in the PBGS reaction mechanism (14).

Because yeast PBGS can bind 16 Zn(II) per octamer (4), which is twice as much as human PBGS (3), a homology model for human PBGS must accommodate this difference in stoichiometry. Our current model contains ZnA and ZnB bound to each of four subunits, whereas the other four subunits contain no metal ions (3). A picture of the Zn(II) sites of the human PBGS model is presented in Fig. 1, where adjacent amino acids are seen as ligands to ZnA (His-131) and ZnB (Cys-132). We propose that this is the basis for communication between the two metal sites.

To test this model, the current work addresses the stoichiometry and function of ZnA and ZnB by use of mutagenesis to remove (or incapacitate) each of the two sites to create the...
mutants here called MinusZnA and MinusZnB. The former is designed to bind only four ZnB, and the latter is designed to bind only four ZnA. We present a kinetic characterization of MinusZnA in comparison to the wild type human PBGS proteins K59, N59, K59/C162A, and N59/C162A, the latter of which is the parent of MinusZnA and MinusZnB. The C162A mutation was introduced to avoid a non-native disulfide and is shown to be benign. Less comprehensive data are presented for the relatively inactive MinusZnB. This work shows that Pb(II) can interact with human PBGS in at least two different ways, consistent with the interpretation of the yeast PBGS crystal structure complexed with Pb(II). However, interaction of Pb(II) with a ZnA ligand could not have been predicted from the crystal structure.

**EXPERIMENTAL PROCEDURES**

**Materials**—Most chemicals and buffers were obtained from Fisher Scientific or Sigma in the purest available form. 2-Mercaptoethanol (βME) was from Fluka and vacuum-distilled prior to use. High purity KOH was from Aldrich. Concentration devices, originally sold under the Amicon label, were obtained from Fisher as were Slide-A-Lyzer dialysis cassettes, originally sold under the Pierce label. Atomic absorption standards were SpecPure grade and obtained from Alfa Aesar.

**Protein Expression and Purification**—Human PBGS and mutants thereof were expressed and purified from an artificial gene as described previously (3). The mutations were achieved using the QuikChange technology of Stratagene. The sense strand primers were GCCCTAGC-CTAACCGCATCGACGATTGCCCGCTTCG (for C162A), CCTG- TGGCTGGCCTTCCACACCATCTCCAAGTGCACGGCGCATC- TCC (for C122A, C124A, C125A), CGTTAGCTTCCAGGCTTGGTCC- GTGTGTCGGAGC (for H131A), and GGCGGCGGCGCGCCGCTAG- CTGCG (for C223A). All resultant plasmids were sequenced throughout the coding region by the sequencing technology of Stratagene. The sense strand primers were GCGTATG-

**Pb(II) Inhibition Studies**—Fixed-time lead inhibition assays included the holoenzymes as purified and were carried out with no added metals, with 20 μM Pb(II), and with both 10 μM Zn(II) and 20 μM Pb(II) added to the assay preincubation mixture. Because of the ability of phosphate to buffer metal ions, these assays were carried out in TES-KOH at an initial pH of 7.0. Free Pb(II) is <20 μM due to complexation by βME. After the addition of ALA to 10 mM, the active proteins were assayed for 5 min fixed time assay; MinusZnB was assayed for 5 h. For determination of K_a and V_max values, the ALA-HCl concentrations were 10 μM, 30 μM, 100 μM, 300 μM, 1 mM, 3 mM, and 10 mM with stock dilutions made in 0.1 M βME to keep the assay pH and ionic strength constant. All assays were terminated with a one-half volume of STOP reagent (20% trichloroacetic acid, 0.1 M HgCl_2, K_a, and V_max determinations for MinusZnB used 0.5 mg ml⁻¹ and 18-h assays. The catalytic constant K_cat/M in 10 mM Zn(II) was reduced by five orders of magnitude, K_a, and V_max determinations were carried out in the presence and absence of Mg(II) to control for activity due to trace contamination by E. coli PBGS. Mg(II) causes a pH-dependent activation of the E. coli protein (16), but not the human protein. Porphobilinogen formed was determined by absorbance at 555 nm about 5 min after the addition of a one- and one-half volume of modified Ehrlich’s reagent. The extinction coeffi- cient of the pink complex formed (Δε_555) was 62 000 cm⁻¹ M⁻¹. The C162A mutation was prepared with the intention of reducing the relatively inactive MinusZnA in comparison to the wild type human PBGS protein (3). Following a brief (2 h) dialysis back into neutral pH buffer (0.1 M TES-KOH, 10 mM βME, pH 7.0), the proteins were analyzed for Zn(II) content by atomic absorption spectroscopy and assays for total Zn(II) content in the presence of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 μM Zn(II) added to the assay. For all data points, A_555 values were ≥0.10 and thus of high confidence.

**RESULTS**

**Protein Expression and Purification**—Previous work showed that human PBGS corresponding to the N59 allele partitioned into the soluble fraction of E. coli 3-fold better than K59 (3). The C162A mutation was prepared with the intention of removing an internal disulfide bond with Cys-119. This disulfide was apparent in a model of human PBGS based on the yeast PBGS crystal structure (3) and is seen in preliminary crystal-
lographic data on bovine and human PBGS. 2 The resulting protein, which is named N59/C162A, partitioned almost completely into the soluble fraction of the E. coli host cell and exhibited purification properties as described for Asn-59 (3). The mutant proteins MinusZnA and MinusZnB also expressed very well, partitioned into the soluble fraction of the host cell, and purified like the wild type proteins. K59/C162A was found to partition like K59 where the predominant fraction was in inclusion bodies (3). As before, all proteins were purified from the soluble fraction. The final purified pools from the S-300 column were analyzed for Zn(II) content by atomic absorption spectroscopy. Wild type variants N59, K59, N59/C162A, and K59/C162A contained 8 Zn per octamer, MinusZnA purified with 3.9 ± 0.6 Zn/octamer, and MinusZnB purified with 4.0 ± 0.5 Zn/octamer. DEAE fractions of MinusZnB that contained trace activity that can be activated by Mg(II) indicated E. coli PBGS contamination, and this MinusZnB was further purified by a second pass through the DEAE column, and once again, the activity activated by Mg(II) eluted solely in the tail end of the protein peak. Because high level heterologous expression in E. coli can lead to incomplete N-terminal processing, the purified protein was digested with trypsin and subjected to MALDI-TOF mass spectral analysis. No evidence was found for aberrant N-terminal processing. The N-terminal methionine was present with complete removal of the formyl group (data not shown).

Catalytic Activities of Human PBGS Variants—The N59/C162A and K59/C162A variants of PBGS as well as MinusZnA are all enzymatically active with specific activities comparable with N59 and K59 (3) under standard assay conditions (0.1 M potassium phosphate, final pH 6.8, 10 μM Zn(II), 10 mM βME). The specific activities at pH 6.8 are constant from 1 to 30 mM Zn(II) to prevent slow through some alternate mechanism that is in-

| pH | K59 | N59 | K59/C162A | N59/C162A | MinusZnA | MinusZnB |
|----|-----|-----|----------|----------|----------|----------|
| Kₘ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ | Vₘₐₓ |
| 6  | 1.4  | 43  | 1.2  | 26  | 2.6  | 32  | 26  | 28  | 0.17 | 18  | 19  |
| 7  | 0.09 | 44  | 0.18 | 21  | 0.17 | 36  | 0.17 | 28  | 0.10 | 18  | 20  |
| 8  | 0.04 | 23  | 0.014 | 5   | 0.04 | 25  | 0.05 | 14  | 0.03 | 9   | NA  |

*NA, not assayed.

* Values adjusted for trace contamination with E. coli PBGS as determined by a second set of assays done in the presence of 1 mM Mg(II) (raw data Kₘ = 8.5 mM and Vₘₐₓ = 0.004).

* Substrate activation was observed when [ALA] > 0.04 μM.

**-determining ALA in a

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2 H. L. Carrell, unpublished results.
The catalytic Zn(II) and its cysteine ligands.

**Stoichiometry of Zn(II) Required for Optimal Activity**—Bovine and human PBGS (K59) require at least four Zn(II) per octamer for full activity (3, 6, 7, 11). The activity is not increased when eight Zn(II) per octamer are present. To determine the Zn(II) dependence for N59/C162A and Minus ZnA, substoichiometric amounts of Zn(II) were added to assays containing 2 μM PBGS subunits, which first had been depleted in Zn(II) by pH 5.0 dialysis (<0.2 Zn(II)/octamer). Back-dialysis to pH 7 resulted in an enzyme with one to two Zn(II)/octamer. For this reason, the rates with no additional Zn(II) were in the range of 25–50% of the maximal activity as illustrated in Fig. 2B. In all three cases, near maximal activity was obtained after addition of approximately two Zn(II) per octamer. The slope of the line through these low Zn(II)/octamer points shows that four Zn(II) per octamer corresponds to 100% activity. The Zn(II) in the assay components was determined to be negligible. These data are presented in Fig. 2B alongside a complete Zn(II) titration for human PBGS N59 (3).

**The Effect of Lead**—Pb(II) inhibition of PBGS has historically been attributed to Pb(II) replacing Zn(II). The preparation of Minus ZnA and Minus ZnB variants allowed us to determine whether these Zn(II) sites are related to Pb(II) inhibition. We compared the ability of Pb(II) to inhibit the activities of wild type human PBGS variants, Minus ZnA, and Minus ZnB using a variety of different assays. Table II shows that, for the holoenzymes of K59, N59, K59/C162A, and N59/C162A (eight Zn(II)/octamer), with no Zn(II) added to the assay, 20 μM Pb(II) in the preincubation mixture caused significant inhibition in a 5-min assay (15–37% activity). This can be effectively counteracted by the addition of 10 μM Zn to the preincubation mixture (80–99% activity). Minus ZnA was also sensitive to inhibition by Pb(II) (56% activity) but significantly less than the wild type variants as measured using the fixed time 5-min assay. This reduced inhibition for Minus ZnA suggests the ligands to ZnA play a role in the inhibition of human PBGS by lead. Continuous assays, as described below, revealed the basis for the decreased sensitivity of Minus ZnA to lead inhibition relative to the other active proteins.

Pb(II) inhibition of human PBGS cannot be thoroughly assessed with simple fixed-time assays, because continuous assays with Pb(II) show a nonlinear time dependence as seen before for bovine PBGS (24). This suggests that the way in which Pb(II) binds to PBGS varies depending on the presence or absence of substrate or product as seen for other slow binding inhibitors. The nonlinearity of human PBGS activity in the presence of lead is shown using continuous assays (carried out at 20 μM and 50 μM Pb(II)) following the accumulation of porphobilinogen by its absorbance at 236 nm. For the wild type human PBGS variants studied, K59, N59, and N59/C162A, the nonlinear time dependence was observed. The absorbance data for N59 is illustrated in Fig. 2C. The initial velocities, steady-state velocities, and half-times are reported in Table III; the velocity data are reported as percentage activity relative to a control assay without Pb(II). For each variant, with Pb(II) the apparent Vₘₐₓ values at neutral pH. Despite the non-

![Image](95x401 to 252x730)

**Fig. 2.** A, the pH-dependent kinetic behavior of human PBGS, using N59 as an example, pH 6 (□), pH 7 (○), pH 8 (●). B, Zn(II) activation of Zn-depleted human PBGS N59 (○); N59/C162A (●); Minus ZnA (□). Previously published data (3) on N59 (●) is included to illustrate Zn(II) saturation. The linear regression through all data at less than two added Zn(II) per octamer is identical to that previously obtained for N59 (●). C, porphobilinogen production time course of N59 in the presence of Pb(II), respectively; the respective apparent Kₘ values are given in parenthesis. The preparation of Minus ZnA and Minus ZnB using a variety of different assays. Table II shows that, for the holoenzymes of K59, N59, K59/C162A, and N59/C162A (eight Zn(II)/octamer), with no Zn(II) added to the assay, 20 μM Pb(II) in the preincubation mixture caused significant inhibition in a 5-min assay (15–37% activity). This can be effectively counteracted by the addition of 10 μM Zn to the preincubation mixture (80–99% activity). Minus ZnA was also sensitive to inhibition by Pb(II) (56% activity) but significantly less than the wild type variants as measured using the fixed time 5-min assay. This reduced inhibition for Minus ZnA suggests the ligands to ZnA play a role in the inhibition of human PBGS by lead. Continuous assays, as described below, revealed the basis for the decreased sensitivity of Minus ZnA to lead inhibition relative to the other active proteins.

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| Protein | Assay components | control | 20 μM Pb | 10 μM Zn 20 μM Pb |
|---------|------------------|---------|----------|-------------------|
| K59    |                  | 100 ± 4 | 17 ± 7   | 80 ± 3            |
| N59    |                  | 100 ± 5 | 15 ± 12  | 90 ± 3            |
| K59/C162A |              | 100 ± 4 | 30 ± 6   | 99 ± 3            |
| N59/C162A |              | 100 ± 1 | 37 ± 1   | 88 ± 1            |
| MinusZnA |                  | 100 ± 1 | 56 ± 1   | 91 ± 2            |
| MinusZnB |                  | 100 (96.8) | 88 (98.5) | 94 (94.8)         |

*6-h assays with time points at 30-min intervals giving rates of ΔA₃₆₅ increases of ~0.025 OD h⁻¹. Linear regression R values are given in parenthesis.
tions of Pb(II) cause a progressive increase in the $K_m$ value for ALA as well as a progressive decrease in the $V_{max}$ value. If one presumes that the Pb(II) replaces Zn(II), then this supports a role for Zn(II) in both substrate binding and catalysis.

Pb(II) Competition for the Zn(II) Sites of Human PBGS—Purified human and bovine PBGS contain eight Zn/octamer (3, 7). When dialyzed against phosphate buffer at neutral pH at 4°C in the absence of additional Zn(II), four tightly bound Zn(II) remain (3, 12). Assuming these Zn(II) are available to bulk solution, the estimated $K_d$ for this Zn(II) is $<0.1 \mu M$; a second group of four Zn(II) are shown to be in equilibrium with free Zn(II) and bind with a $K_d$ of about 5 $\mu M$. If instead, the protein is first brought to pH 5, all enzyme-bound Zn(II) are free to dissociate (3). Low pH appears to remove the enzyme’s asymmetry; a subsequent equilibrium dialysis at room temperature in neutral pH phosphate buffer revealed the binding of eight Zn(II) per octamer with a single $K_d$ of about 1 $\mu M$ (3). Preliminary data on the ability of Pb(II) to displace the Zn(II) of human PBGS suggested that Pb(II) preferentially displaces Zn(II) from only half of the Zn(II) sites (3). Here, equilibrium dialysis experiments were performed on N59/C162A, MinusZnA, and MinusZnB using asymmetric “as purified” protein in the absence of substrate. These data reflect Pb(II) and Zn(II) occupancy at $t = 0$ of the kinetic studies, prior to the hysteresis shown in Fig. 2C. Fig. 3 illustrates Zn(II) binding to N59/C162A, MinusZnA, and MinusZnB at 10 $\mu M$ Zn(II) and 0.5 $\mu M$ Pb(II) in the presence and absence of 20 $\mu M$ Pb(II). These data show that, at 0.5 $\mu M$ Zn(II), Pb(II) competes favorably for the ZnB sites (see MinusZnA results). Results with MinusZnB show far less ZnB (II) binding to the ZnA site, and this enzyme-bound Zn(II) is not displaced by Pb(II).

**DISCUSSION**

The Two Zn(II) Sites of Human PBGS—The model for Zn(II) bound to human PBGS presented in Fig. 1 contains two different ligand environments for Zn(II) and pertains to one-half the human PBGS subunits. An alternative model would have one ZnA on each of four of the subunits and one ZnB on each of the other four subunits. It is not yet possible to differentiate between these two models for the asymmetric eight Zn(II)-containing forms of human PBGS. Nevertheless, the Zn(II) stoichiometry data presented here on MinusZnA and MinusZnB establishes important aspects of our model; one-half the Zn(II) purified with human PBGS are ZnA, the other half are ZnB, and the ligands to these Zn(II) are likely as depicted in Fig. 1. The activity data for MinusZnA and MinusZnB unequivocally establish ZnB as the catalytically essential four Zn(II). This agrees with popular dogma, but was not obvious on the basis of common Zn(II)-containing enzymes. ZnA, inferred from the EXAFS to be pentacoordinate with predominantly oxygen and nitrogen ligands (8), appeared to be a good candidate for a catalytic Zn(II), because its coordination environment resembles many well characterized catalytic Zn(II) (25). Furthermore, an EXAFS study showed four ZnA to bind more tightly than four ZnB to bovine PBGS (8). This agreed with the notion that ZnA is the essential Zn(II) but is contrary to chemical intuition and to the crystallographic data on yeast PBGS (14). ZnB, which is inferred from the EXAFS to have a tetrahedral array of cysteine ligands, closely resembles structural Zn(II) such as is found in zinc fingers (25). There are, however, emerging examples of catalytically essential Zn(II)-thiolates (26). Based on a comparative analysis of the structures of yeast and *Pseudomonas aeruginosa* PBGS, the locations of both ZnA and ZnB appear to be within the active site when a mobile flap covering the active site is closed (1). The yeast PBGS crystal structure shows ZnB to be at high occupancy, and the low activity of the MinusZnB variant of human PBGS establishes ZnB as the one that is of catalytic importance. The role of ZnA in protein function, if any, remains unclear.

**Lead Interactions with PBGS—Mammalian PBGS has long been known as a target enzyme for lead poisoning, as a Zn(II) metalloenzyme, and as an enzyme in which cysteine residues in a reduced state are important for activity. An attractive hypothesis developed, which presumes that there is a catalytically essential Zn(II) bound to these cysteine residues and that Pb(II) inhibits the protein through replacement of this Zn(II) (27). We now see that there are two types of Zn(II) in human PBGS, and at least one cysteine function in binding each of the Zn(II) types. The current study defines the relationships of these Zn(II) and their ligands to inhibition by Pb(II).**

The lead inhibition studies presented here and previously indicate that the interaction of Pb(II) with mammalian PBGS is altered in the presence of substrate (24). Furthermore, the steady-state inhibited rate was attained slowly. This type of phenomenon has been referred to as “slow binding inhibition” and indicates that the interaction of the inhibitor, here Pb(II), with the enzyme changes as a consequence of protein motion during catalysis. One precedent for a substrate-induced change in metal binding properties of a PBGS is the substrate-induced disproportionation of Mn(II) between two sites of *Bradyrhizobium japonicum* PBGS (28). We propose that the structural
amino acids are different for PbB Cys-132 as ligands, but the side-chain configurations of these subunit at the same time. This is because both use Cys-124 and mutually exclusive, that is, they cannot exist on the same important to notice that Pb(II) occupancy of these two sites is configurations of Cys-122, Cys-124, Cys-132, and Cys-223 were tetrahedron. To model the PbAB site, the side-chain rotamer conformations relative to A. The programs Molscript and Raster3D (36, 37) were used to prepare the figure.

basis for nonlinear kinetics of Pb(II)-inhibited human PBGS derives from a change in the dynamic distribution of Pb(II) between multiple binding sites before and during turnover. The fact that MinusZnA does not exhibit time-dependent reaction kinetics in the presence of Pb(II) (see Table III) implicates the mutated ZnA ligands in one of the Pb(II)-binding sites. Because the steady-state rates are not altered, the ZnA ligands are not implicated in the complex formed during the steady-state inhibition but rather in a tight-binding inhibitory Pb(II) site, which preferentially exists in the absence of substrate. This site is disfavored in the presence of substrate; it is missing in MinusZnA.

We modeled the Pb(II)-binding sites in human PBGS (Fig. 4), drawing on published observations of Pb(II) bound to yeast PBGS in two alternate positions (2). One Pb(II) site appears to use the three ZnB ligands. This Pb(II) is called PbB and is shown in Fig. 4A. The three ligands are Cys-122, Cys-124, and Cys-132, and the lone pair of electrons on Pb(II) occupies the fourth position of a tetrahedral coordination geometry. Because the distance between Zn(II) and sulfur is typically around 2.2 Å and Pb(II) to sulfur distances are ~2.9 Å (29), PbB does not occupy precisely the same location as ZnB but is slightly further away from the cysteines. The second site is called PbAB; it is proposed to use Cys-124 and Cys-132 but not Cys-122 (numbered as in human PBGS), where the side chains are in a different configuration not seen in the yeast PBGS structure (2, 30). We assume that Cys-223 is the ZnA ligand most likely to interact with Pb(II) in the alternate configuration. PbAB is illustrated in Fig. 4B. PbAB uses as ligands the side chains used by both ZnA and ZnB, Cys-124, Cys-132, and Cys-223, and the lone pair from Pb(II) sits at the fourth position of the tetrahedral. To model the PbAB site, the side-chain rotamer configurations of Cys-122, Cys-124, Cys-132, and Cys-223 were sampled while the backbone coordinates were held fixed. It is important to notice that Pb(II) occupancy of these two sites is mutually exclusive, that is, they cannot exist on the same subunit at the same time. This is because both use Cys-124 and Cys-132 as ligands, but the side-chain configurations of these amino acids are different for PbB versus PbAB. Pb(II) is apparently inhibitory in both the PbB and the PbAB sites for two reasons. First, in both sites the lone electron pair on Pb(II) would interfere sterically with substrate or product binding. Second, both positions for Pb(II) disallow the binding of ZnB, which this study shows is catalytically important perhaps through interaction with its cysteine ligands.

One might ask why turnover disfavors the PbAB site. The cysteine residue analogous to Cys-223 of human PBGS showed itself in the yeast PBGS crystal structure (Protein Data Bank file law5) to be the first ordered residue after a disordered region (14). The P. aeruginosa PBGS crystal structure identified this disordered region as a flap that can isolate the active site from solvent during catalysis (31). Our comparison of the two available structures of yeast PBGS (Protein Data Bank codes law5 and 1lyv) using the combinatorial extension method (32) shows the cysteine to be positioned differently when the lid is ordered relative to when it is disordered. Consequently, we propose that motion of the active site lid during catalysis moves Cys-223 and disfavors the population of the PbAB. Thus, at steady state, the enzyme-bound Pb(II) is preferentially bound to the PbB site.

Variants K59 and N59 in Lead Poisoning—A body of epidemiological literature correlates the allelic variation between K59 and N59 with a differential susceptibility to lead poisoning (e.g. Ref. 33). A smaller number of epidemiological studies do not support this hypothesis (e.g. Ref. 34). Prior studies on purified K59 and N59 did not reveal a molecular basis for a difference nor did they support the existence of a difference (3). The current study reveals a small difference in the half-time for recovery from Pb(II) inhibition during turnover conditions in vitro. However, because most PBGS in circulating erythrocytes is left over from the massive heme biosynthesis that occurs in reticulocytes (that is, catalysis is not occurring), this difference is unlikely to affect the interaction of Pb(II) with blood PBGS, which is the major site of Pb(II) binding in circulating blood (35).

Conclusion—We have prepared the mutant human PBGS proteins MinusZnA and MinusZnB, which each purify with four Zn(II) per octamer, thus supporting the model of native human PBGS as normally purifying with four ZnA and four ZnB. This metal stoichiometry is fundamentally different from that of yeast PBGS, which was previously used as a model system for the human enzyme in lead poisoning. The mutagenesis studies show that ZnB and its ligands are catalytically important, whereas ZnA and its ligands are not. This provides biochemical characterization and confirmation of an unusual catalytic Zn(II) site. Pb(II) bound at a site analogous to the ZnB site (PbB) is shown to be a site of lead inhibition during steady-state turnover. Pb(II) is proposed alternatively to bind to a PbAB site, which uses ligands normally involved in the binding of both ZnA and ZnB. The PbAB site is disfavored during turnover. In both environments, it is the lone election pair of Pb(II) that is proposed to alter the active site environment and disfavor substrate binding and subsequent catalysis.

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