Human porphobilinogen synthase (PBGS) can exist in two dramatically different quaternary structure isoforms, which have been proposed to be in dynamic equilibrium (Breinig, S., Kervinen, J., Stith, L., Wasson, A. S., Fairman, R., Wlodawer, A., Zdanov, A., and Jaffe, E. K. (2003) Nat. Struct. Biol. 10, 757–763). The quaternary structure isoforms of PBGS result from two alternative conformations of the monomer; one monomer structure assembles into a high activity octamer, whereas the other monomer structure assembles into a low activity hexamer. The kinetic behavior of these oligomers led to the hypothesis that turnover facilitates the interconversion of the oligomeric structures. The current work demonstrates that the interactions of ligands at the enzyme active site promote the structural interconversion between human PBGS quaternary structure isoforms, favoring formation of the octamer. This observation illustrates that the assembly and disassembly of oligomeric proteins can be facilitated by the protein motions that accompany enzymatic catalysis.

A recent description of a rare human allele of porphobilinogen synthase (PBGS) revealed that the enzyme can exist as two alternate quaternary structures wherein the oligomeric state is dictated by distinctly different structures for their component crystallographic asymmetric unit (1). The monomer is an αβ-barrel protein with a 23-residue N-terminal arm. The octameric form is comprised of four “hugging dimers” (Fig. 1a), whereas the hexameric form is comprised of three “detached dimers” (Fig. 1b). In both oligomeric assemblies there are invariant barrel-to-barrel subunit interactions within the dimers and quasi-equivalent arm-to-bottom-of-barrel subunit interactions between adjacent dimers (Fig. 1). However, the octamer has a unique “hugging” arm-to-barrel interaction that is not seen in the hexamer.

Preparations of heterologously expressed wild type human PBGS predominantly contain octamer, but there is a small propensity to adopt the hexameric structure (1). In contrast, heterologous expression of the rare human variant, F12L, yields protein that purifies as the hexamer and does not readily take on the octameric structure. The homo-octameric wild type and homohexameric F12L forms of human PBGS exhibit dramatic differences in pH activity profile and in the kinetic $K_m$ values (Table I) (1) despite the fact that amino acid residue 12 does not interact directly with active site residues in either quaternary structure isomorph (Fig. 1). However, the arm-to-barrel interface found in the hugging dimer provides substantial stabilization for the solvent-accessible face of the αβ-barrel wherein lies the active site. This region of the protein, which is disordered in the hexamer, includes an ~11-aminic acid stretch that normally serves to gate the active site from solvent, also called the active site lid. With the active site more solvent-accessible in the hexamer, a high external pH is required to drive the Schiff base formation that is necessary for catalysis (2); this contributes to the high pH required for F12L activity. The decreased affinity of F12L for substrate is attributed to the loss of interactions between the destabilized active site lid and the carbonyl moiety of the outermost substrate (3).

Coexpression of wild type and F12L (WT+F12L) yielded a mixture of hetero-octamers and heterohexamers, each of which contained some Phe and some Leu (1). The characterization of these hetero-octamers and hetero-octamers of WT+F12L demonstrated that the quaternary structure, and not the specific amino acid mutation, was responsible for the differences in $K_m$ values and pH activity profiles (1, 2). The coexpressed hetero-oligomers of WT+F12L could be separated by ion exchange chromatography and/or by native gel electrophoresis. The mobilities of wild type and F12L are used, respectively, as standards for octameric and hexameric hetero-oligomers. According to these analytical tools, the hetero-oligomers are stable structures that repeatedly chromatograph/electrophorese true. However, determination of $K_m$ and $V_{max}$ values for the hetero-oligomers fit best to a model (Equation 1) wherein catalysis is by an equilibrium of the octameric (low $K_m$ or $K_{m1}$) and hexameric (high $K_m$ or $K_{m2}$) forms of the hetero-oligomers (Table I). In fact, studies with wild type human PBGS at non-physiologic conditions (pH 9) also fit best to catalysis by a dynamic equilibrium of low $K_m$ (octameric) and high $K_m$ (hexameric) forms.

$$\text{velocity} = \frac{V_{max}[S]}{K_m1 + [S]} + \frac{V_{max}[S]}{K_m2 + [S]} \quad (\text{Eq. 1})$$

Although the thermodynamic foundation for the preference of wild type human PBGS and F12L to assemble into octamer and hexamer, respectively, remains unclear, the current study capitalizes on this differential preference to evaluate factors that effect the interconversion of quaternary structure isoforms of WT+F12L. The current work substantiates the hypothesis that the hetero-oligomers of human PBGS can interconvert, as shown in Fig. 2, upon addition of substrate. This is the first time such a dynamic structural rearrangement has been demonstrated. The current work exploits the different kinetic, chro-
matographic, and electrophoretic properties of the hexamers and octamers and utilizes dynamic light scattering to determine the factors that catalyze the interconversion of PBGS quaternary structure isoforms.

PBGS from any organism has not been observed in a form smaller than the dimer. Thus heteromeric WT/H11001 F12L proteins are believed to be made up of stable dimers of three compositions (which are at position 12): Phe+Phe, Phe+Leu, and

FIG. 1. Crystal structures of octameric human wild type PBGS (Protein Data Bank code 1E51) and the hexameric F12L variant (Protein Data Bank code 1PV8). a, the monomer that forms the octamer (upper left corner) of human PBGS. The product-like intermediate at the active site is shown in space-filling presentation and colored red. The active site lid is shown as a ribbon in red. Phe12 is in space-filling presentation and colored in the Corey-Pauling-Koltun color scheme. The subunits of the hugging dimer (upper right corner) are colored cyan and purple. In this hugging dimer, an N-terminal arm of each subunit is wrapped around the αβ-barrel of the adjacent subunit to form the arm-to-barrel interface (within the orange oval). The arm-to-bottom-of-barrel interface (within the dark blue oval) between two hugging dimers is shown in the tetramer configuration (lower right corner). This interface is between an arm of one cyan subunit and the barrel of the purple subunit of the adjacent dimer. The octamer includes four dimers, each rotated 90° around the central axis. b, the monomer that forms the hexamer of F12L is shown in yellow (upper left corner); the putative reaction intermediate is shown in red space-filling presentation. Leu12 is shown in space-filling presentation and colored in the Corey-Pauling-Koltun color scheme. The active site lid is disordered and not seen in the crystal structures of F12L. The configuration of the N-terminal arm of F12L is dramatically altered, and the arm-to-barrel interface that is in the hugging dimer (a) is not seen in the detached dimer (upper right corner). Addition of a second detached dimer (lower right corner) involves an arm-to-bottom-of-barrel interaction (within the dark blue oval) between the N-terminal arm of the blue subunit of one dimer and the barrel of the yellow subunit of the second dimer. This arm-to-bottom-of-barrel interaction is quasiequivalent to that shown for the octameric assembly. The hexamer (lower left corner) of F12L involves three detached dimers arranged at a 120° rotation angle around the central axis.
Leu+Leu. As some heterologously expressed human PBGS partitions to inclusion bodies, one cannot assume that the ratio of these dimers is 1:2:1 in the soluble isolated protein. However, because the homomeric Phe12-containing protein folds and assembles preferentially as the octamer and the homomeric Leu12-containing protein folds and assembles exclusively as the hexamer, one can propose that Phe+Phe dimers will preferentially assemble to the octamer and that Leu+Leu dimers will preferentially assemble to the hexamer. This is qualitatively consistent with the observed Phe:Leu ratio of the hetero-oligomers reported previously (1) and confirmed herein. Following this rationale, one can also propose that a dynamic reequilibration of heteromeric oligomers (Fig. 2) would result in a disproportionation reaction that favors accumulation of Phe12 in the octamer and Leu12 in the hexamer. The reported results substantiate this hypothesis.

**Experimental Procedures**

**Genes and Constructs**—The human PBGS N59/C162A is used as WT in this study (4). The F12L variant was constructed as previously reported (1). The plasmid (pET17b-WTF12L) containing one promoter has been reported previously (1). The plasmid with two promoters (pET17b-WTPF12L) was constructed as follows. The pET17b vector was modified from pET17b. A point mutation at position 3249 (T

**TABLE I**

**Kinetic parameters of homomeric and heteromeric human PBGS**

The $K_m$ and $V_{\text{max}}$ values were obtained by varying [ALA] from 3 $\mu$M to 10 mM and were calculated by fitting the kinetic data to Equation 1. $K_m$ and $V_{\text{max}}$ (both mM) are interpreted as the $K_m$ values for the octamer and hexamer, respectively. The reported $V_{\text{max}}$ values (mM $\cdot$ min $^{-1}$ $\cdot$ mg $^{-1}$) reflect the mole fraction of quaternary species under assay condition.

|          | F12L | WT† | WT+F12L Pool I | WT+F12L Pool II |
|----------|------|-----|----------------|-----------------|
| $K_m$    | pH 7 | 0.25 ± 0.01 | 0.47 ± 0.07 | 0.19 ± 0.01 |
| $V_{\text{max}}$ | pH 7 | 55.5 ± 0.2 | 2.98 ± 0.38 | 13.06 ± 0.40 |
| $K_m$    | pH 7 | 17.7 ± 1.1 | 7.34 ± 1.53 | 5.05 ± 0.96 |
| $V_{\text{max}}$ | pH 7 | 1.14 ± 0.05 | 6.60 ± 0.23 | 9.05 ± 0.37 |
| $V_{\text{max}}$ | pH 9 | 0.015 ± 0.001 | 0.69 ± 0.15 | 0.05 ± 0.01 |
| $K_m$    | pH 9 | 8.16 ± 0.13 | 2.99 ± 0.80 | 1.89 ± 0.25 |
| $V_{\text{max}}$ | pH 9 | 4.6 ± 0.1 | 4.46 ± 0.80 | 4.17 ± 1.80 |
| $V_{\text{max}}$ | pH 9 | 18.2 ± 0.2 | 2.74 ± 0.66 | 4.78 ± 0.19 |

a Data were taken from Ref. 1.

b The previous reported (1) $K_m$ at pH 9 for WT was 0.35 ± 0.09; this was a misprint that represented the fit to the Hill equation.

course activity assay at $t = 0$, ALA-HCl was added to a bulk assay volume (30 ml), and 1 ml of assay mixture was withdrawn at desired time points into a tube containing 0.5 ml of STOP reagent. The stopped assay solutions were vortexed vigorously and then centrifuged for 3 min to remove the precipitated protein and 2-mercaptoethanol. A pink-colored complex was formed by mixing 0.8 ml of assay solution with 0.8 ml of Ehrlich’s reagent. The color was allowed to fully develop for 8 min before the determination of absorbance at 555 nm ($e_{555} = 60,200 \text{ M}^{-1} \text{ cm}^{-1}$) (5).

**Equilibrium Dialysis Experiments**—The dialysis buffer was 0.1 M BTP-HCl at desired pH values, 10 mM 2-mercaptoethanol, and 10 $\mu$M ZnCl$_2$. The reported pH values reflect the dialysis buffer pH after the addition of ALA (where included). Protein solutions (~200 $\mu$g at 3–7 $\mu$g/ml) were dialyzed in the presence or absence of ALA against 300 ml of buffer at 37 °C for 24 h or longer under gentle agitation (50–60 rpm) in an air shaker. Samples of the buffer were periodically withdrawn for determination of porphobilinogen concentration using Ehrlich’s reagent (see above). Samples were also taken from the dialysis cassette at desired time points for native gel electrophoresis. Gels were then scanned, and the fractional intensity of the protein bands at each time point was analyzed using SigmaGel™ gel analysis software (Jandel Corp.).

**Native Gel Electrophoresis**—Native gel electrophoresis was done on a PhastGel system (Amersham Biosciences). Samples were prepared by mixing the protein solution with native gel running buffer (0.1 M Tris/HCl, pH 8.8, 20% glycerol, 0.0025% bromphenol blue) to reach a final protein concentration of ~1 $\mu$g/ml. Four microliters of each sample solution was loaded on a homogeneous 12.5% polyacrylamide gel in 0.1 M Tris-glycine buffer (pH 9) with 0.05 M ZnCl$_2$. The gel was run with PhastGel native buffer strips (Amersham Biosciences, 0.88 M l-alanine, 0.25 M Tris, pH 8.8, made of 3% Agarose IEF). After separation, gels were developed on the PhastGel system using Coomassie staining.

**Light-scattering Measurements**—The molecular weight change during substrate-induced dynamic interconversion was monitored using a temperature-controlled DynoPro dynamic light-scattering instrument (Protein Solutions Inc.) at 37 °C.

**Mass Spectral Analysis of the Disproportionation of Heteromeric Oligomers**—WT+F12L Pool I and Pool II protein solutions underwent 24 h of equilibrium dialysis in the presence of 10 mM ALA. The hexameric and octameric forms of the protein were separated after the dialysis using Mono-Q column. The Mono-Q buffer was 30 mM potassium phosphate, pH 7.0, 10 mM 2-mercaptoethanol, 10 $\mu$M ZnCl$_2$. The hexamer and octameric forms were separated using a 0.02–1.0 $\mu$M KCl gradient in 27 column volumes. Fractions containing hexameric and octameric forms were pooled and concentrated to a final concentration of ~1 mg/ml. The concentrated pools were dialyzed against 300 ml of 2 mM BTP-HCl buffer at pH 7.0 for 3 h to remove the phosphate from the Mono-Q buffer. Samples were subject to overnight trypsin (Promega,}

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sequencing grade modified) digestion using a 1:20 (w/w) trypsin:protein ratio. The tryptic peptide mixtures were spotted on a gold plate with cyanogen bromide matrix on top of that. The mass spectral data were collected using Reflex IV matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Bruker Inc.).

Quaternary Structure Equilibration in the Presence of Irreversible Inhibitors—Enzyme (−3 mg/ml) was preincubated at 37 °C in normal assay buffer for 10 min prior to the addition of inhibitors, succinylacetone or 4,7-dioxosebacic acid (4,7-DOSA) and levulinic acid to final concentrations of 1, 0.1, and 10 mM, respectively. The reactions proceeded at 37 °C. Protein samples were taken at desired time points for native gel electrophoresis analysis.

RESULTS
Preparation of Heteromeric Oligomers of WT+F12L—Wild type human PBGS and the F12L variant were coexpressed from the same plasmid with a T7 promoter upstream of each gene. The coexpressed protein WT+F12L separates into two distinct pools of PBGS activity on either DEAE or Q-Sepharose anion-exchange columns. The pools were further purified on an S-300 column (1). The Q-Sepharose (or DEAE) pool that elutes at lower ionic strength is called Pool I and is comprised of heterohexamers. In the current preparation heterohexamers were made up of 31% Phe12 and 69% Leu12. The pool that elutes at higher ionic strength is called Pool II and is comprised of hetero-oligomers. In the current preparation hetero-oligomers were made up of 67% Phe12 and 33% Leu12. Earlier preparations in which wild type human PBGS and F12L were coexpressed under one promoter just upstream of the wild type gene showed that Pool I contained 49% Phe12 and 51% Leu12, whereas Pool II contained 71% Phe12 and 29% Leu12 (1). The dependence of the distribution of Phe12 and Leu12 on the structure of the expression plasmid is unclear because the current work shows that disproportionation between quaternary isoforms will occur during expression and accumulation as long as substrate is present in the cell.

Interconversion and Reequilibration of WT+F12L Heteromeric Oligomers as Monitored by Equilibrium Dialysis—Using heteromeric Pool I and Pool II, equilibrium dialysis was used to provide a physical demonstration that substrate can promote the reequilibration between octamer and hexamer according to the reaction illustrated in Fig. 2. The proteins comprised of Pool I and Pool II were separately dialyzed at 37 °C in the presence and absence of the substrate, ALA, and the quaternary structure was probed by native gel electrophoresis as a function of dialysis time. Product formation was monitored in the dialyze and indicated that catalysis was ongoing throughout the dialysis procedure (Fig. 3a). The electrophoretic results (Fig. 3b) show that the oligomeric structures are stable for at least 46 h when dialysis is carried out in the absence of substrate. However, the presence of the substrate causes a dramatic reequilibration of quaternary structure isoforms, favoring accumulation of the octamer in all cases (Pool I and Pool II at pH 7.0 and pH 9.0). These pH values correspond to the optimal pH for the activity of the octamer and the hexamer, respectively (1). Densitometry was used to estimate the rate and extent of the reequilibration reaction for Pool I at pH 7 and pH 9 and for Pool II at pH 9 (Fig. 3c) where the data were fitted to an exponential function and fit to rate constants of 0.54 h−1 (Pool I, pH 7), 0.4 h−1 (Pool I, pH 9), and 1.8 h−1 (Pool II, pH 7), respectively. The rate and extent of conversion of Pool I to octamer was greater at pH 7 than at pH 9, suggesting that there is a pH dependence to the equilibrium between the PBGS quaternary structure isoforms. This is consistent with data on wild type human PBGS (octamer) where kinetic evidence indicates catalysis by both octamer and hexamer at pH 9 but catalysis only by octamer at pH 7 (1).

Analysis of Pool I and Pool II Oligomers following the Substrate-induced Interconversion of Quaternary Isoforms—Using freshly prepared hetero-oligomers of WT+F12L, the Pool I and Pool II proteins were separated chromatographically and then dialyzed for 24 h at pH 7 in the presence of 10 mM ALA. Following dialysis, the reequilibrated hetero-oligomers were again separated into their hexameric and octameric components by chromatography on a Mono-Q column. The separated proteins were characterized for their specific activity and for their Phe12 and Leu12 content. The specific activities of hexamer and octamer are dramatically different with hexamer showing optimal activity at pH 9, and the activity of octamer is maximal at pH 7 (Table II) (1). A native gel of these proteins shows the relative distribution of octamer and hexamer in samples before dialysis and after Mono-Q chromatography (Fig. 4a). Following chromatographic separation, the proteins were called Pool I hexamer, Pool I octamer, Pool II hexamer, and Pool II octamer and are described below. The peak to elute first on the Mono-Q column runs comparably to the F12L mutant, which is the hexamer. The hexameric structure is substantiated by native gel electrophoresis and by specific activity at pH 7 and pH 9 (Fig. 4a and Table II). The second peak to elute runs comparably to the WT human PBGS, which is the octamer. The octameric structure is substantiated by native gel electrophoresis and by specific activity at pH 7 and pH 9 (Fig. 4a and Table II). Consistent with the documented characteristics of human PBGS hexamers and octamers (1), Pool I hexamer and Pool II hexamer have very low activity at pH 7 and considerable activity at pH 9. Pool I octamer and Pool II octamer both show substantially higher activity at pH 7 than at pH 9.

To address the hypothesis that hetero-oligomeric WT+F12L pools will disproportionate, resulting in accumulation of Phe12 in the octamer and Leu12 in the hexamer, the Phe12 and Leu12 content of the heteromeric PBGS oligomers was determined before dialysis and after Mono-Q chromatography. The proteins were subjected to tryptic digestion, and the N-terminal peptide was analyzed by laser desorption mass spectroscopy. Prior work had established that this technique gives quantitative results that are comparable to those obtained by quantitative N-terminal sequencing for these two very similar peptides (1). The masses of the N-terminal tryptic peptides are...
observed when the Pool II protein, initially at 67:32, was dialyzed against ALA. On a native gel, Pool II shows a major octamer band and a light hexamer band (Fig. 4a). After 24 h of dialysis in the presence of ALA, the Pool II octamer was enriched in Phe (Phe:Leu ratio of 81:19), and the small amount of remaining hexamer was enriched in Leu (Phe:Leu ratio of 14:86). The mass spectral data unequivocally establish the disproportionation of heteromeric PBGS isoforms under turnover conditions. The mass spectral data also confirm that human PBGS with phenylalanine at position 12 prefers the octamer, which assembles from the hugging dimer, and protein with leucine at position 12 prefers the hexamer, which assembles from the detached dimer. It remains unclear whether the dynamic structural interconversion illustrated in Fig. 2 is further complicated by the dissociation of dimers into their component monomers. Such a reequilibration of dimers could result in the disproportionation of a population of Phe+Leu dimers into Phe+Phe and Leu+Leu dimers.

**Dynamic Interconversion of WT**

**F12L Hetero-oligomeric PBGS Quaternary Structure Isoforms Is Accompanied by an Increase in Activity at pH 7—** Prior kinetic studies of PBGS showed that the dependence of activity on substrate concentration does not follow a simple hyperbolic Michaelis-Menten relationship (Table I). The kinetic data are best fit using a double hyperbolic equation (Equation 1), which is applicable to a model in which two enzymes of different kinetic parameters are catalyzing the same reaction (7). The substrate-induced structural interconversion (Figs. 3 and 4) and the resulting specific activity changes (Table II) suggest that the heteromeric WT+F12L pools undergo structural rearrangement under assay conditions. Since substrate-induced interconversion favors the octamer, which has increased activity at pH 7 relative to the hexamer, then the time course of product formation should show an increase in rate with time. This change is proposed to be most dramatic for Pool I (the heterologously expressed hetero-hexamer of WT+F12L) at pH 7 where the difference between the activities of octamer and hexamer is the greatest. As predicted, the specific activity of WT+F12L Pool I protein increases with time (Fig. 5a). The rate constant (1.05 h⁻¹) is based on fitting the data to an exponential rate equation and is about twice as fast as that determined from the equilibrium dialysis experiment (0.54 h⁻¹), qualitatively consistent with an expected delay caused by the time required for substrate and product to cross the dialysis membrane. Similar behavior was observed for WT+F12L Pool II where the rate constant is 7.9 h⁻¹ (Fig. 5b).

**Structural Interconversion Monitored by Light-scattering Measurements—** Structural interconversion between hexamer and octamer not only has an effect on the activity of the protein; it also alters the average molecular weight of protein. Using dynamic light scattering, the molecular weight change during the structural rearrangement of Pools I and II in the presence of substrate was monitored (Fig. 5). The light-scattering data yield rate constants that are comparable to the rate constants from the time course activity assay (1.0 h⁻¹ for Pool I at pH 7 and 5.0 h⁻¹ for Pool II), thus supporting the conclusion that the increase in activity is due to the interconversion of hexamer to octamer in the presence of substrate.

**Turnover Is Not Required for the Structural Interconversion of PBGS Quaternary Structure Isoforms—** The studies presented above demonstrate that turnover promotes the formation of octameric PBGS from hexameric PBGS but do not address whether turnover is required. To address the question of whether active site ligand binding and/or Schiff base intermediate formation is sufficient to promote interconversion, we looked at three active site-directed inhibitors, 4,7-DOSA (8),
succinyl acetone (9), and levulinic acid (8). Native gel electrophoresis demonstrates that the interaction of human PBGS with all three inhibitors facilitates the formation of octamer from heterohexameric Pool I (Fig. 6), although the interconversion rates are much slower than those seen with substrate. Levulinic acid has been reported as a competitive inhibitor for PBGS proteins from many species including *E. coli*, yeast, and pea (8). Succinyl acetone is also a commonly used inactivator of PBGS and has physiologic significance in the inactivation of human PBGS for patients with hereditary tyrosinemia (10, 11). 4,7-DOSA and succinyl acetone (10) are active site-directed irreversible inhibitors, and both of them have been observed by x-ray crystallography to be covalently bound though Schiff base linkage to PBGS from *E. coli* and yeast, respectively (3, 9); *E. coli* and yeast PBGSs are mechanistically similar to human PBGS in that they require an active site catalytic zinc ion that is important to the binding and reactivity of the *Km*-determining substrate molecule (2, 12). 4,7-DOSA has been reported as a very potent irreversible inhibitor for human PBGS (3). Kinetic data show that enzyme inactivation was almost complete after 4 h at the concentrations of 4,7-DOSA and succinyl acetone used in the experiment shown in Fig. 6 (data not shown). However, the relative intensity of the octamer band shows a significant increase between 4 and 24 h of reaction. This observation indicates that turnover is not required for the dynamic interconversion of PBGS quaternary structure isoforms, but active site ligand binding and perhaps also Schiff base formation are required.

**DISCUSSION**

**Physiologic Relevance of the Interconversion of PBGS Quaternary Structure Isoforms**—PBGS catalyzes the first common reaction in the biosynthesis of the tetrapyrrole pigments such as heme, chlorophyll, phycobilins, siroheme, corrin, and cofactor F430 (11, 13). These diverse energy-modulating cofactors are essential for all but the most specialized cellular life forms. The photoreactive nature of tetrapyrrole biosynthetic intermediates dictates that tetrapyrrole biosynthesis is tightly regulated, particularly in plants. For plant PBGS, it has been proposed that the allosteric magnesium (which is not present in the metazoan or yeast proteins (12)) mediates the interconversion of PBGS quaternary structure isoforms, but active site ligand binding and perhaps also Schiff base formation are required.

**TABLE II**

|                | F12L Pool I | WT Pool I | WT+F12L Pool I | Pool I hexamer | Pool I octamer | Pool II hexamer | Pool II octamer |
|----------------|-------------|-----------|----------------|----------------|----------------|----------------|----------------|
| pH 7           | 0.3         | 56.9      | 7.9            | 21.8           | 0.5            | 11.5           | 0.2            |
|                | 14.5        | 13.1      | 6.6            | 5.4            | 5.8            | 6.8            | 1.6            |
| pH 9           |             |           |                |                |                |                |                |

**FIG. 5.** Human PBGS WT+F12L substrate-induced dynamic interconversion (disproportionation) at pH 7 monitored by an activity time course (**a**) and dynamic light scattering (**b**). **a**, WT+F12L Pool I; **b**, WT+F12L Pool II.

**FIG. 4.** Analysis of the disproportionation products of human WT+F12L Pool I and Pool II. **a**, native gel electrophoresis of the pools before dialysis, after 24 h of dialysis against ALA at pH 7, and after chromatographic separation on the Mono-Q column. **b**, mass spectral data (MS) for the N-terminal tryptic peptide of WT+F12L Pool I and WT+F12L Pool II before and after the disproportionation reaction and Mono-Q chromatography. WT and F12L are included as standards.
Interconversion of Protein Quaternary Isoforms

PBGS proteins that require the allosteric magnesium, the magnesium binding site is located at the arm-to-barrel interface of the hugging dimer. This binding site is absent in the hexamer (1, 3, 14, 15). Previously reported data showed that addition of magnesium favors the largest form (octameric) during native gel electrophoresis (14). The binding of magnesium stabilizes the hugging dimer through interactions with an arginine residue on the N-terminal arm of one subunit and a glutamic acid residue from the αβ-barrel of the opposite subunit. Other N-terminal arm residues then interact with residues on the active site lid thus stabilizing a closed lid configuration. This motion of closing the active lid is a possible structural basis for active site ligands promoting octamer formation.

In humans, however, tetrapyrrole biosynthesis is regulated predominantly by the availability of the PBGS substrate ALA (16), and there is no evidence that the slow equilibration of PBGS quaternary structure isoforms plays a regulatory role under normal physiologic conditions. However, human PBGS is a primary molecular target for the environmental toxin lead. Lead has been deduced to bind at the active site of human PBGS in a manner similar, but not identical, to the catalytic zinc (4). Binding of lead in this fashion would destabilize an interaction between Cys223 of one subunit and Phe12 of the other subunit of the hugging dimer and thus might favor formation of the hexameric assembly. With this in mind, it is noted that substrate-induced recovery of PBGS from lead inhibition has been documented as a slow reaction with a half-time of 7 min (4). One cannot help but wonder whether accumulation of the hexameric form of human PBGS plays a role in lead poisoning.

The Relationship between Protein Motion, the PBGS-catalyzed Reaction Mechanism, and Interconversion of PBGS Quaternary Structure Isoforms—PBGS catalyzes an asymmetric condensation of two molecules of ALA to form the monopyrrole porphobilinogen. There are probable mechanistic differences between those PBGS that use a catalytic zinc and those that do not (2, 12), and the following discussion is specific to the zinc-utilizing PBGS protein from humans. The first ALA to bind forms a Schiff base intermediate between its C-4 and the glutamic acid residue on the opposite subunit (2, 12). The second substrate then binds loosely to the second substrate binding site in the structural interconversion. Schiff base formation to bind the second ALA molecule (9, 12), and the following discussion is specific to the zinc-utilizing PBGS protein from humans. The first ALA to bind forms a Schiff base intermediate between its C-4 and the glutamic acid residue on the opposite subunit (2, 12). The second substrate then binds loosely to the second substrate binding site in the structural interconversion.

Light Scattering as a Method for Monitoring the Equilibration of Quaternary Structure Isoforms—Light scattering has been used as a powerful tool for protein characterization, including purification monitoring (23), aggregation (24), assembly (25), structural stability (26), and crystal growth (27). The current work is the first use of light scattering to monitor the molecular weight change due to the dynamic interconversion of quaternary structure isoforms of a homomeric protein. This demonstration opens possibilities for investigation of the kinetics of protein structure changes that in some cases could be very difficult to monitor by other spectroscopy techniques.

The Dynamic Interconversion of PBGS Quaternary Isoforms and the Resulting Disproportionation Introduce the Morpheein Concept of Quaternary Structure Equilibrium—The morpheein concept describes alternate quaternary structures of a protein with different functional characteristics, such as the octameric and hexameric forms of PBGS. The differences in oligomeric multiplicity, structure, and function result from a dramatic conformational change in the monomer. The interconversion and disproportionation between heteromeric human PBGS morpheeins, as demonstrated herein, substantiates the reaction illustrated in Fig. 2. This type of protein structure change has been proposed to provide the structural basis for allosteric regulation of plant PBGS where an allosteric magnesium ion binds to the arm-to-barrel interface that is in the hugging dimer/octamer but not in the detached dimer/hexamer (1). Although PBGS comprises a prototype morpheein system, there are other systems described in the literature for which the morpheein concept appears to be applicable. One such system is a tetramer to hexamer equilibrium proposed for mammalian ribonucleotide reductase (28, 29).

FIG. 6. Dynamic interconversion of WT+F12L Pool I at pH 7 in the presence of the inhibitors succinyl acetone, 4,7-DOSA, and levulinic acid as monitored by native gel electrophoresis.

| Time | Reaction Products |
|------|------------------|
| 1 hr | + Succinyl acetone + 4,7-DOSA + Levulinic acid |
| 2 hr | + Succinyl acetone + 4,7-DOSA + Levulinic acid |
| 3 hr | + Succinyl acetone + 4,7-DOSA + Levulinic acid |
| 4 hr | + Succinyl acetone + 4,7-DOSA + Levulinic acid |

The interconversion of quaternary structure isoforms, such as the octameric to hexameric form of human PBGS, has been shown to be regulated by light scattering. This method has been used as a powerful tool for protein characterization, including purification monitoring and the study of aggregation, assembly, structural stability, and crystal growth. The current work is the first use of light scattering to monitor the molecular weight change due to the dynamic interconversion of quaternary structure isoforms of a homomeric protein. This demonstration opens possibilities for investigation of the kinetics of protein structure changes that in some cases could be very difficult to monitor by other spectroscopy techniques.

The morpheein concept describes alternate quaternary structures of a protein with different functional characteristics, such as the octameric and hexameric forms of PBGS. The differences in oligomeric multiplicity, structure, and function result from a dramatic conformational change in the monomer. The interconversion and disproportionation between heteromeric human PBGS morpheeins, as demonstrated herein, substantiates the reaction illustrated in Fig. 2. This type of protein structure change has been proposed to provide the structural basis for allosteric regulation of plant PBGS where an allosteric magnesium ion binds to the arm-to-barrel interface that is in the hugging dimer/octamer but not in the detached dimer/hexamer (1). Although PBGS comprises a prototype morpheein system, there are other systems described in the literature for which the morpheein concept appears to be applicable. One such system is a tetramer to hexamer equilibrium proposed for mammalian ribonucleotide reductase (28, 29).
as that seen here for human PBGS, may be a common phenomenon in protein conformational changes. We propose that this sort of interconversion may interfere with the growth of diffraction quality protein crystals. For instance the first PBGS crystals were published in 1975 (30), but the first structure did not appear until 1997 (31). The rapid interconversion of PBGS quaternary structure isoforms (morphoforms) becomes apparent for some PBGS, like the plant PBGS, in a dramatic protein concentration dependence of the specific activity (32). We propose that this rapid interconversion accounts for our failure to obtain diffraction quality crystals for plant PBGS. The ability of PBGS proteins to accommodate a variety of oligomeric forms may also relate to its high thermal stability (33, 34). It is possible that other homo-oligomeric proteins from mesophilic organisms that show unusually high thermal stability do so because they can accommodate more than one “native” structure.

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