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Review

Allostery and the dynamic oligomerization of porphobilinogen synthase

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ABSTRACT

The structural basis for allosteric regulation of porphobilinogen synthase (PBGS) is modulation of a quaternary structure equilibrium between octamer and hexamer (via dimers), which is represented schematically as 8mer $\Longleftrightarrow$ 2mer $\Longleftrightarrow$ 2mer$*$ $\Longleftrightarrow$ 6mer$*$. The "$*$" represents a reorientation between two domains of each subunit that occurs in the dissociated state because it is sterically forbidden in the larger multimers. Allosteric effectors of PBGS are both intrinsic and extrinsic and are phylogenetically variable. In some species this equilibrium is modulated intrinsically by magnesium which binds at a site specific to the 8mer. In other species this equilibrium is modulated intrinsically by pH with the guanidinium group of an arginine being spatially equivalent to the allosteric magnesium ion. In humans, disease associated variants all shift the equilibrium toward the 6mer$*$ relative to wild type. The 6mer$*$ has a surface cavity that is not present in the 8mer and is proposed as a small molecule allosteric binding site. *In silico* and *in vitro* approaches have revealed species-specific allosteric PBGS inhibitors that stabilize the 6mer$*$. Some of these inhibitors are drugs in clinical use leading to the hypothesis that extrinsic allosteric inhibition of human PBGS could be a mechanism for drug side effects.

Introduction

Porphobilinogen synthase (PBGS)[1] is an ancient metabolic enzyme responsible for the biosynthesis of the fundamental monopyrrole that is the building block for myriad tetrapyrrolic cofactors required for life (porphyrin, chlorophyll, vitamin B12, siroheme, phytobilin, cofactor F430, etc.) [1]. The PBGS catalyzed reaction is an asymmetric condensation of two molecules of the substrate 5-aminolevulinic acid (ALA). All organisms that carry out respiration, photosynthesis, or methanogenesis require PBGS, and the enzyme is remarkably conserved throughout evolution [2]. The metabolic pathway from porphobilinogen to the tetrapyrroles is phylogenetically variable but universally populated by photoreactive intermediates whose accumulation can be toxic [3,4]. Thus, as part of the control of tetrapyrrole biosynthesis, PBGS evolved an allosteric regulation mechanism. Unexpectedly, PBGS allostery involves alternate, functionally distinct multimeric assemblies whose architectures are so different as to require multimer dissociation as part of the interconversion between active and inactive assemblies [5]. Novel aspects of the allosteric regulation of PBGS led us to define a morpheein model of allostery (described elsewhere [6]); proteins that use this allosteric mechanism display properties that are characteristically different from proteins that follow the classic Monod–Wyman–Changeux and Koshland–Nemethy–Filmer models [7]. Herein, we describe the phylogenetically variable allosteric regulation of PBGS; presumably these variations evolved due to the considerably different environments that organisms inhabit and the phylogenetic variation in the subcellular location of PBGS. In most species PBGS is located in the cytosol [8,9]. However, in plants it is in the chloroplast [10], and in apicomplexan parasites it is in the apicoplast [9].

An overview of PBGS structure and allostery

PBGS is encoded by a single gene and each PBGS multimer is composed of multiple copies of the same protein. Each PBGS subunit consists of a ~300 residue αβ-barrel domain, which houses the enzyme active site in its center, and a >25 residue N-terminal arm domain (Fig. 1) [11]. Allosteric regulation of PBGS can be described in terms of the orientation of the αβ-barrel domain with respect to the N-terminal arm domain [5]. These two domains undergo a dramatic reorientation around a hinge region in the transition between active and inactive multimers (Fig. 1). Like most αβ-barrel enzymes, substrate access to the active site is mediated by a mobile loop that serves as an active site lid; the lid of each subunit comes from the same subunit as the rest of the active site. Allosteric regulation of PBGS can be described in terms of intersubunit interactions that assist in stabilizing an ordered conformation of the active site lid [12]. These interactions involve the N-terminal arm domain from a subunit that is different from the subunit contributing the active site. In the active multimeric

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[1] *Abbreviations used:* PBGS, porphobilinogen synthase; ALA, 5-aminolevulinic acid; SEC, size exclusion chromatography.

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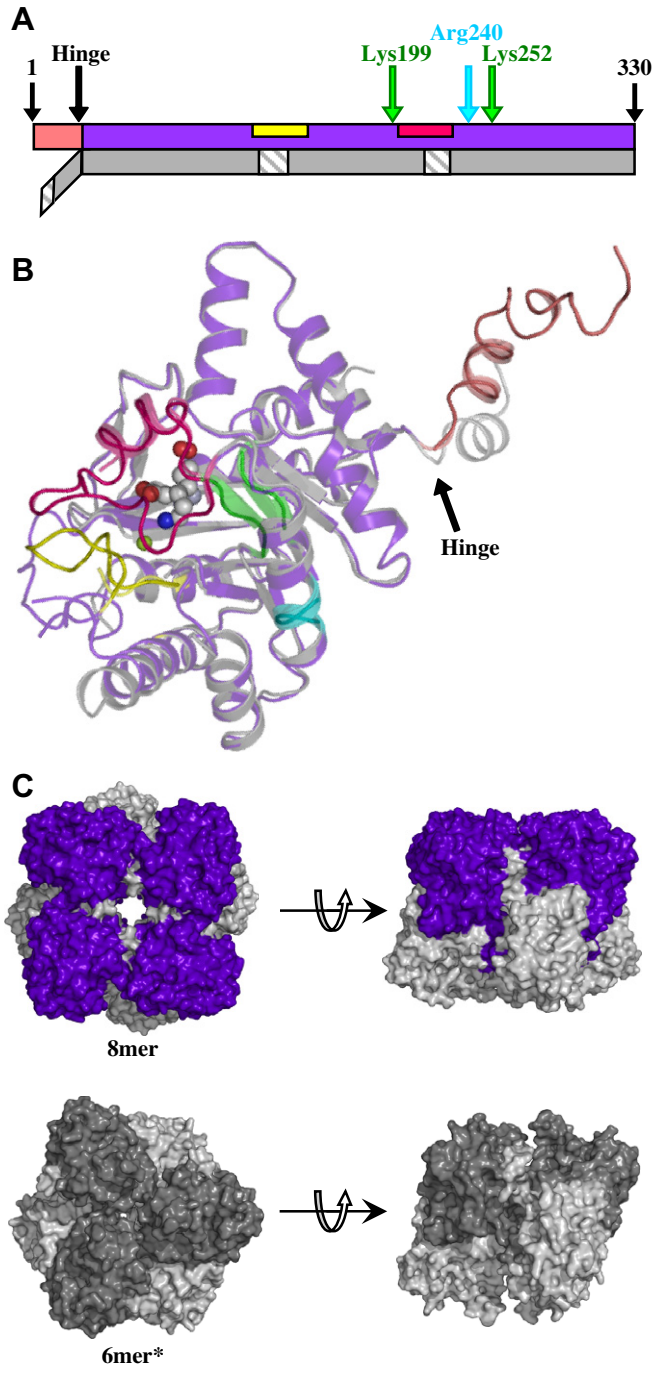

Fig. 1. Structural overview of PBGS. (A) Schematic of PBGS structural features numbered as for human PBGS. The upper bar represents wild-type human PBGS with the αβ-barrel in purple and the N-terminal arm in salmon. Yellow and red bars highlight an active site metal binding region and the active site lid. Conserved lysine residues interacting with the substrates and an arginine responsible for quaternary structure maintenance are indicated in green and cyan. The lower (gray) bar represents the naturally occurring F12L variant of human PBGS, which has similar tertiary structure, but a dramatically different orientation of the N-terminal arm relative to the αβ-barrel. Regions of disorder in the F12L structure are highlighted with dashed gray lines. (B) Overlay of a subunit of wild-type human PBGS (pdb: 1E51) and the F12L variant (pdb: 1PV8) colored as in A. The product porphobilinogen (CPK coloring with the carbons in white) and the active site zinc (colored lime) are shown as spheres. (C) Top and side views of human PBGS octamer (8mers) in purple and white, and hexamer (6mer∗s) in gray and white shown as surfaces.

state, the N-terminal arm of one subunit interacts with the αβ-barrel domains of two other subunits. Similarly, each αβ-barrel interacts with the N-terminal arm of two adjacent subunits. One of

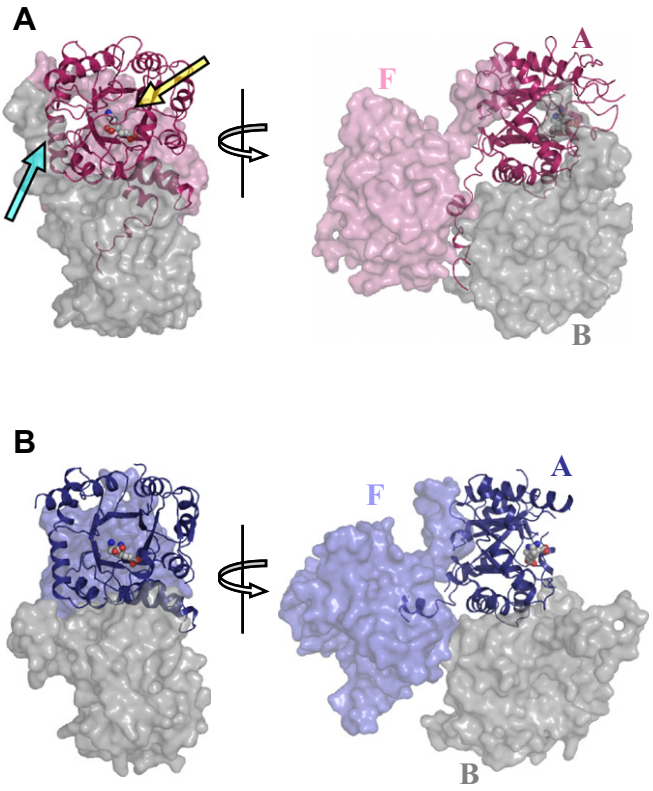

Fig. 2. Front and side views of the A, B and F subunits of the active PBGS octamer (8mer) (pdb: 1E51) and inactive hexamer (6mer∗) (pdb: 1PV8) crystal structures. (A) Subunit A of the PBGS octamer is shown as a dark pink cartoon with porphobilinogen (spheres, colored CPK with carbons in gray) bound in the active site. The active site lid is highlighted by the yellow arrow. Subunits B and F of the octamer are shown as transparent surfaces in gray and light pink, respectively. The lid-stabilizing arm-to-barrel interface (exclusive to the 8mer) is highlighted by the cyan arrow. (B) Subunit A of the PBGS hexamer is shown as a dark blue cartoon with a product intermediate (spheres, colored CPK with carbons in gray) bound in the active site. The active site lid is disordered in the hexamer structure. Subunits B and F of the hexamer are shown as transparent surfaces in gray and light blue, respectively.

these interactions helps to stabilize a "closed" conformation of the active site lid; this is the A/B interaction of Fig. 2. The other interaction restricts solvent access from the other end of the αβ-barrel; this is the A/F interaction in Fig. 2. In the inactive multimeric state, the N-terminal arm domain is not involved in the lid-stabilizing A/B subunit–subunit interaction. In the crystal structure of the inactive assembly, the active site lid is disordered (see Figs. 1 and 2B).

As described above, allosteric regulation of PBGS fits a familiar theme; the transition between active and inactive forms involves a conformational change that affects the traffic of ligands into and out of the enzyme active site. The initially unexpected aspect of PBGS allostery is that the active multimer is an octamer (8mer), the inactive multimer is a hexamer (6mer∗), and the transition between 8mer and 6mer∗ requires dissociation to a dimer. The dimer exists in an equilibrium of conformations, one of which can assemble to the 8mer, another of which can assemble to the 6mer∗. The shorthand equation for this phenomenon is 8mer $\iff$ 2mer $\iff$ 2mer∗ $\iff$ 6mer∗, where the oligomer-determining conformational change (2mer $\iff$ 2mer∗) is a reorientation of the N-terminal arm domain of each subunit with respect to the αβ-barrel domain of the same subunit (seen most clearly in Fig. 1B). Steric considerations prevent this reorientation from occurring in either the 8mer or 6mer∗, thus dissociation is an essential part of the allosteric mechanism.