

Morpheins – A New Pathway For Allosteric Drug Discovery

Eileen K. Jaffe*

Institute for Cancer Research, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA

Abstract: The morphein model of allosteric regulation can be applied as a novel approach to the discovery of small molecule allosteric modulators of protein function. Morpheins are homo-oligomeric proteins where, under physiological conditions, the oligomer can dissociate, the dissociated units can change conformation, and the altered conformational state can reassociate to a structurally and *functionally* distinct oligomer. This phenomenon serves as a basis for allostery, as a basis for conformational diseases, as a basis for drug discovery, and may be applicable to personalized medicine such as in the prediction of drug side effects. Each of these relationships has been established for the prototype morphein, porphobilinogen synthase, where the conformational disease is a porphyria and the drug application is in antimicrobial discovery. These data are presented along with a discussion of other drug targets for which the morphein model of allostery may apply. Such targets include HIV integrase, TNF α , β -tryptase, and p53.

Keywords: Allostery, porphobilinogen synthase, morphein.

INTRODUCTION

Proteins carry out the bulk of the physical and catalytic work of the cell. Errors in protein function provide a basis for disease and, consequently, control of protein function is a goal in drug development. Although early work in protein crystallography encouraged a view of protein structure as largely static, the 21st century view emphasizes the flexibility of proteins and how protein structure dynamics are essential to protein function. This article focuses on a newly discovered mode of protein *quaternary* structure dynamics that promises both to deepen and broaden our understanding of protein structure-function relationships and our ability to control protein function.

Allostery provides a mechanism for functional control of proteins, specifically enzymes, which bypasses the inherent limitations of active site targeting [1]. Allosteric regulators control enzyme function through binding to a site other than the active site; allosteric sites are generally less conserved than active sites. Thus, allostery provides a potential mechanism for species-selective inhibition of an essential, but highly conserved enzyme, as might be useful for the development of new antimicrobials. Allosteric regulators also provide for modulation of a specific enzyme among a large class of functionally similar enzymes (e.g. kinases or phosphatases). Hence, therapeutics that function as allosteric regulators are attractive targets for development [2]. This article focuses on morpheins and the morphein model of allosteric regulation as a novel basis for drug discovery.

Morpheins and the Morphein model of Allosteric Regulation

Morpheins are homo-oligomeric proteins that can dissociate, change conformation in the dissociated state, and

then reassemble to an alternate oligomer [3]. In the dissociated state, one conformation dictates assembly to a particular oligomer and the alternate conformation dictates assembly to an alternate oligomer. A dice model, Fig. (1a), is useful for illustrating how a morphein accomplishes its quaternary structure rearrangement. Unlike proteins that can form amyloid (e.g. prions), the conformational change between morphein forms (components of the equilibrium) does not involve changes in the core protein fold. Rather, the conformational change is characterized by a small number of adjustments in backbone and side chain angles that are kinetically feasible under physiologic conditions. Also, unlike amyloid forming proteins, the alternate oligomer stoichiometries are finite.

The alternate quaternary structure assemblies of a morphein equilibrium have alternate functions, each of which is physiologically relevant [3]. Examples of such alternate functions include, but are not limited to: 1) “on” vs. “off” states, 2) states having different interaction partners in a signaling pathway, 3) states having different affinity or specificity for allosteric effector molecules, and 4) states having different “moonlighting” functions. The ability of proteins to participate in such a reversible quaternary structure dynamic provides one way that a limited number of encoded genes can provide a much larger number of functions. Most significantly, the transition between alternate assemblies provides a mode of allostery. The morphein model of allosteric regulation is illustrated in Fig. (1b). The obligate oligomer dissociation step distinguishes this model of allostery from the more traditional concerted Monod-Wyman-Changeux and sequential Koshland-Nemethy-Filmer models [4, 5], although the morphein model is also concerted.

PORPHOBILINOGEN SYNTHASE

The quaternary structure dynamic illustrated in Fig. (1) became known to us through our work with porphobilinogen synthase (PBGs), which is an enzyme that catalyzes the first

*Address correspondence to this author at the Institute for Cancer Research, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA; Tel: 215-728-3695; Fax: 215-728-2412; E-mail: eileen.jaffe@fccc.edu

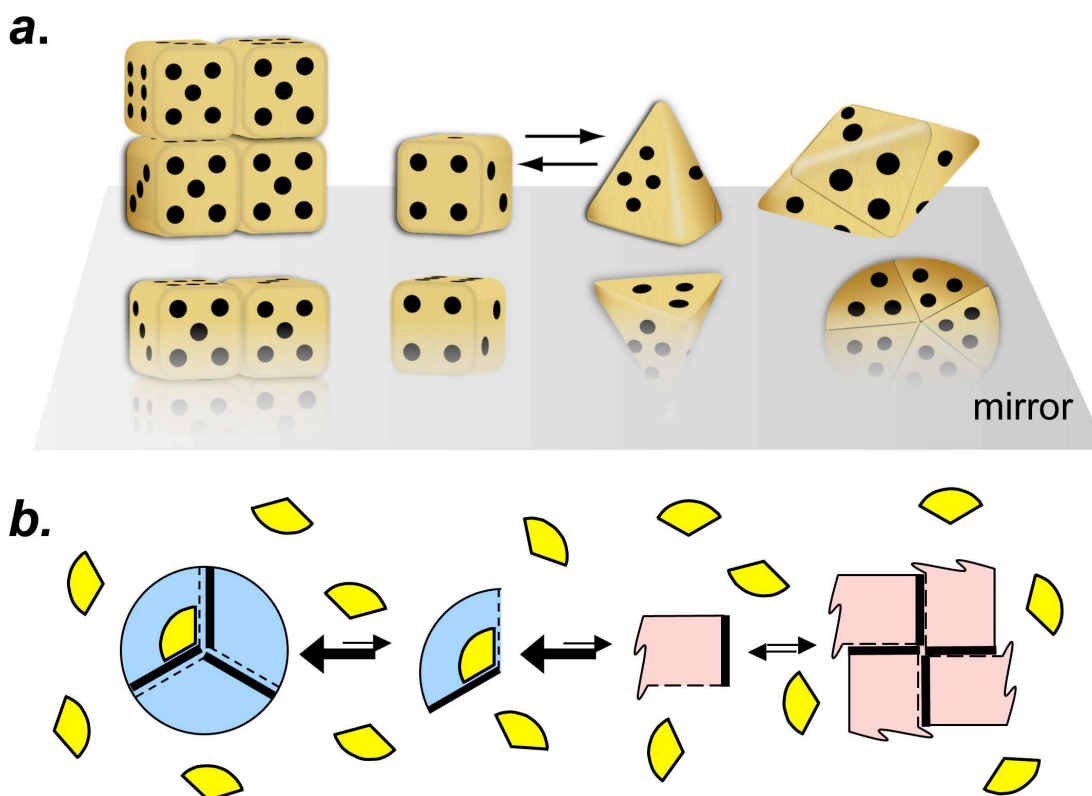


Fig. (1). The quaternary structure dynamic characteristic of morpheeins. **a)** A three dimensional dice analogy is used to illustrate how a die that can morph between a cube and a tetrahedron leads to the assembly of a tetramer and a pentamer respectively. In this case, the common rule of subunit assembly is that “four dots” associate with “one dot”. **b)** The morpheein model of allosteric regulation is illustrated using a two dimensional geometric analogy. A subunit whose conformation is that of a pie wedge (blue) is in equilibrium with a rectangular conformation (pink). The common rule of subunit assembly is that a “dashed line” associates with a “thick line” such that the pie wedge assembles into a circular trimer and the rectangle assembles into a square tetramer. An allosteric regulator, illustrated as a yellow wedge, is shaped to bind preferentially to elements of the trimer. Regulator binding stabilizes the blue components of the equilibrium and draws the equilibrium in the direction of the trimer.

common step in the tetrapyrrole biosynthetic pathway (e.g. heme, chlorophyll, vitamin B₁₂). Our extensive and innovative characterization of the PBGS family of enzymes has taught us that the assembly state is of the utmost importance to PBGS activity and that there are significant phylogenetic differences between the amino acids that govern the equilibrium of PBGS assemblies [6-11]. Although the active site is not at a subunit interface, there are intersubunit interactions that contribute to the structure and dynamics of an active site lid and thus effect catalytic activity [10]. In the case of PBGS, the kinetics and thermodynamics of the quaternary structure equilibria are phylogenetically variable because many of the amino acids that make up the subunit interfaces are not conserved. For PBGS of different species, the quaternary structure equilibrium serves different functions and can be exploited for different purposes. It is a basis for allostery, a basis for a conformational disease, a basis for drug discovery, and may be applicable to personalized medicine in the prediction of drug side effects. The PBGS quaternary structure equilibrium is described below, and the varied aspects of PBGS structure and function are discussed.

The Structure of PBGS

PBGS exists in a dynamic equilibrium of high activity octamers and low activity hexamers, and the interconversion

of these two oligomers involves a conformational change at the level of the dimer. This quaternary structure dynamic is illustrated in Fig. (2), where the stoichiometry is different from Fig. (1a), but the mechanism is the same [3, 8, 9, 11-17]. Oligomers with subtle sequence differences and the technique of mass spectrometry have been used to definitively establish the dissociative mechanism in the hexamer to octamer conversion process [17]. Native polyacrylamide gel electrophoresis, dynamic light scattering, and kinetic hysteresis have been used to monitor the quaternary structure interconversion process [9, 11, 12, 16-18]. In order for this equilibrium to be physiologically relevant, it is important that hexamer and/or octamer dissociation yield dimers where the newly solvent-exposed surfaces are largely hydrophilic. For PBGS there are two possible mechanisms for oligomer dissociation (Fig. (2)) and based on the hydrophilicity of the exposed surfaces, it appears that PBGS from different species may use different dissociation mechanism.

Allosteric Regulation in PBGS

The allosteric regulation of PBGS by magnesium is physiologically relevant in plants. X-ray crystallography has shown how a naturally occurring allosteric activator,

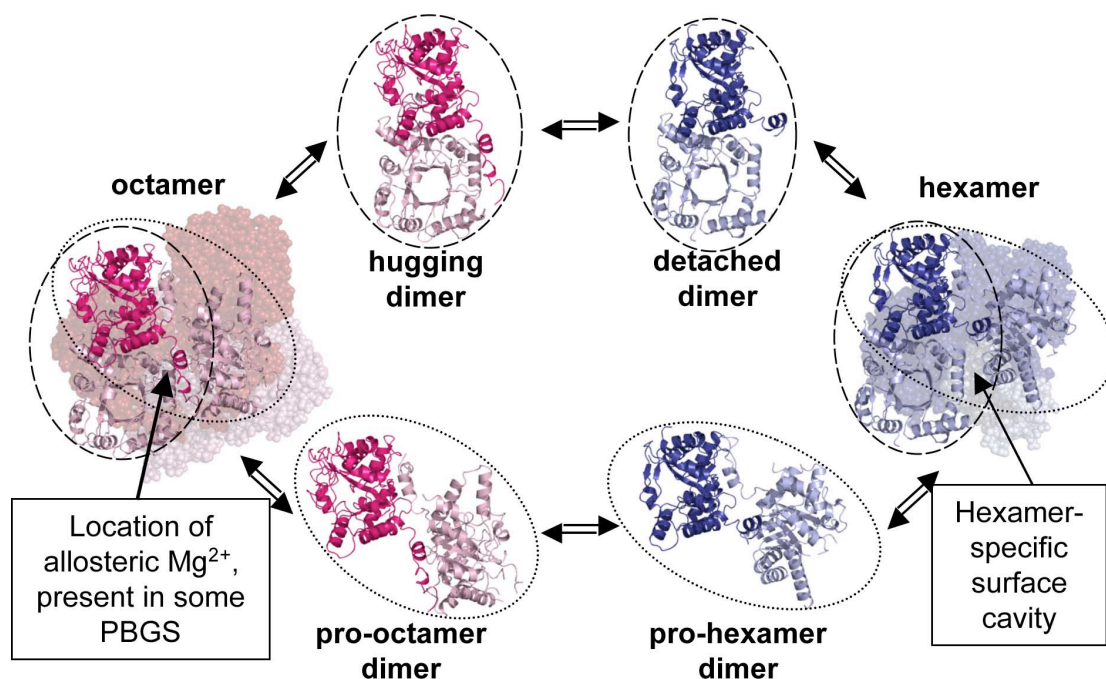


Fig. (2). PBGS structure and quaternary structure equilibrium. Each PBGS monomer consists of an $\alpha_8\beta_8$ -barrel domain, the center of which holds the enzyme active site, and an N-terminal arm domain. Some PBGS also contain N-terminal and C-terminal extensions, not shown here. The top pathway (left to right) shows octamer dissociating to hugging dimers, a conformational change to detached dimers, and assembly to the hexamer. The hugging dimer is the asymmetric unit of many PBGS crystal structures; the N-terminal arm of one subunit “hugs” the $\alpha_8\beta_8$ -barrel of the neighboring subunit. The detached dimer, which does not have the hugging interaction, is the asymmetric unit of the crystal structure of the hexameric human PBGS variant, F12L [11]. The location of one hugging and one detached dimer in the octamer and hexamer respectively are shown using dashed ovals. The bottom pathway shows octamer dissociating to pro-octamer dimers, a conformational change to pro-hexamer dimers, and assembly to the hexamer. The positions of one pro-octamer and one pro-hexamer dimer in the octamer and hexamer respectively are shown using dotted ovals. Conformations that support octamer assembly are shown in shades of red; conformations that support hexamer assembly are shown in shades of blue. Light and dark shades are used for contrast in these homo-oligomeric assemblies.

magnesium, stabilizes the octamer, thus increasing activity [19]. PBGS from photosynthetic eukaryotes contains an allosteric magnesium binding site at a subunit interface that is specific for the high activity octamer. The octamer-specific location of the allosteric magnesium binding site is included in Fig. (2). At low magnesium, plant PBGS, which is located in the chloroplast, exists as the low activity hexamer. Because the intermediates in chlorophyll biosynthesis are highly photo-reactive, it is essential that chlorophyll biosynthesis be tightly controlled such that there is no accumulation of these toxic intermediates. It is well established that one of the first events that occurs when a greening plant is exposed to light is the flooding of the chloroplast with magnesium [20]. The pre-flooding concentration of magnesium is below the K_d of the allosteric magnesium. The post-flooding concentration is saturating for this allosteric magnesium, such that the magnesium influx shifts the equilibrium toward the octamer and contributes to turning on chlorophyll biosynthesis. It is interesting to note that, in addition to plants, the allosteric magnesium binding site is also present in PBGS from nearly all bacteria, archaea, and many unicellular eukaryotes [21]. It is not present in the PBGS from metazoa or fungi. The evolutionary pressure that drove this segregation is not at all clear. Nearly all of those PBGS that do not contain the allosteric magnesium binding site contain instead an arginine residue whose guanidinium group is spatially equivalent to the allosteric magnesium. This arginine also helps stabilize the octamer [16].

Although human PBGS does not contain the allosteric magnesium binding site, it too can be regulated allosterically through oligomer-specific ligand binding. In this case the regulation is caused by small molecule binding to the hexameric assembly, which inactivates the protein. This phenomenon is described below in the context of the now well characterized quaternary structure equilibrium of human PBGS.

Human PBGS Quaternary Structure Equilibrium

Under normal physiologic conditions, wild type human PBGS populates the octameric and hexameric assemblies, with a very small fraction present as the dimer [12]. At basic pH the equilibrium of quaternary structure assemblies is drawn toward the hexamer and this phenomenon is responsible for a pH dependent loss in activity. We determined that the two common alleles for human PBGS have a slightly different propensity to populate the hexameric assembly and asked whether disease associated alleles encode protein with a greater propensity to form hexamer.

The Conformational Disease ALAD-Porphyrria

ALAD-porphyrria is a rare genetic disease whose patients are compound heterozygotes for two of eight known genes that encode defective PBGS proteins [22]. Carriers, who have only one defective gene, have about 50% the normal PBGS activity, but are generally healthy in the absence of

secondary factors. Most of the ALAD-porphyrin associated mutations are not at the enzyme active site. We found that all of the ALAD-porphyrin associated mutations yield proteins that form hexamer more readily than either of the wild type alleles. Hence, one can consider ALAD-porphyrin a conformational disease [15]. We have proposed that diseases caused by a shift in such a quaternary structure equilibrium can broaden the definition of conformational diseases beyond those involving amyloid or other misfolding events [23]. In the case of ALAD-porphyrin, small molecules that stabilize the “healthy” quaternary structure assembly (octamer) may provide a therapeutic advantage. However, the exceedingly rare nature of ALAD-porphyrin argues against PBGS as a viable target for the development of octamer stabilizing therapeutics. Nevertheless, oligomer stabilizing small molecules constitute a viable method to control the function (e.g. activate or inhibit) of proteins that function as morphoeins.

Off-Target Drug Side Effects and Personalized Medicine

We have considered whether there are small molecules in the human environment (foods, drugs, environmental contaminants) that can preferentially bind to and stabilize the human PBGS hexamer and inhibit enzyme activity. The hexameric assembly of PBGS contains an oligomer-specific surface cavity, illustrated in Fig. (2), that is not present in the octamer. Small molecules that bind to this hexamer-specific surface cavity will stabilize the hexamer, draw the quaternary structure equilibrium toward the hexamer, and inhibit enzymatic activity. If such molecules derive from foods, drugs, or environmental contaminants, then these have the capacity to inhibit human PBGS activity and cause physiologic responses reminiscent of porphyria and/or lead poisoning, for which PBGS inhibition is a primary effect. Using a computational docking and an *in vitro* validation approach, we have identified two amebicidal drugs that stabilize the human PBGS hexamer *in vitro* [8]. The side effects of these drugs are consistent with inhibition of human PBGS. Knowledge of which proteins are morphoeins, which drugs alter their quaternary structure equilibrium, and an understanding of how allelic differences effect the quaternary structure equilibrium has promise for application to personalized medicine.

Allosteric Inhibition of PBGS as a Foundation for Antimicrobials and Herbicides

Normally one would not consider the highly conserved and essential tetrapyrrole biosynthetic pathway for antimicrobial or herbicide development. However, we can exploit differences between the components of the quaternary structure equilibrium of PBGS from different organisms and phylogenetic differences in the hexamer-specific surface cavity. For instance, we now know that some PBGS (e.g. human, pea, *Yersinia enterocolitica*) participate in the formation of octamers, dimers, and hexamers, while others (e.g. *Pseudomonas aeruginosa* and *Toxoplasma gondii*) populate only the octameric and dimeric states [7]. Our success in the discovery of species-selective allosteric PBGS inhibitors to date has focused on the hexamer-specific surface cavity [6, 8, 9, 24]. This surface is phylogenetically variable and contains topologies (crevices) that can serve as small molecule binding sites.

Such surface cavities provide opportunities for small molecule allosteric regulation of PBGS activity and a novel approach to PBGS inhibition that avoids targeting the highly conserved enzyme active site. Although we and others have investigated active site directed inhibition of PBGS, potent and species specific active site inhibitors of PBGS from a human pathogen have not been reported [19, 25-31]. Species-selective inhibitors that function by stabilizing the hexamer of pea PBGS and compounds that stabilize the hexamer of *Y. enterocolitica* PBGS were discovered by computational docking of small molecule libraries from Life Chemicals, Inc., to the hexamer-specific surface cavity using the program GLIDE and other components of the Schrodinger software suite [32, 33]. The mechanism of inhibition is established to be *via* hexamer stabilization and human PBGS is not inhibited [9, 24]. Although we have not established that inhibition of PBGS will kill plants or *Y. enterocolitica*, it is established that PBGS inhibition in *T. gondii* can clear the parasite from infected fibroblasts [7], suggesting promise for targeting PBGS in the apicomplexan parasites that cause toxoplasmosis and malaria.

Application of the Morphoein Behavior of PBGS to the Science of Therapeutics

PBGS is the prototype morphoein and though it may only have limited application in the science of therapeutics, its behavior provides a road map for how one might approach other proteins that function as morphoeins.

For instance, it is known that TNF α can be inhibited by a compound that stabilizes a dimer, while TNF α is known to exist primarily as a trimer [34]. It is known that HIV integrase participates in a quaternary structure equilibrium that can be modulated by peptides that have been named shiftides [35, 36]. It is known that β -tryptase exists as functionally distinct tetramers that are slow to interconvert, consistent with a system that must dissociate, change conformation, and reassociate differently [37]. It is known that p53, which is mutated in more than half of all cancers, forms a tetramer when it interacts with DNA, but its core domain can assemble as a trimer in the absence of DNA [38]. This, taken with the knowledge that p53 has important cytosolic functions (where DNA is not present), suggests that some important p53 functions may be as a trimer. Although these proteins are not yet established to function as morphoeins, they, and other proteins, are reported to have behaviors that are consistent with the morphoein view of protein quaternary structure dynamics. The approach of stabilizing one or another of the quaternary structure assemblies that comprise the morphoein equilibrium is proposed to provide a new and exciting approach to understanding and controlling the function of proteins, which is, after all, a primary mode of action for small molecule therapeutics.

SUMMARY

Using PBGS as an example, we show that 1) naturally occurring mutations to may perturb the normal balance of quaternary structure assemblies (with alternate functions) and contribute to disease states; 2) stabilization of one oligomer in an equilibrium of quaternary structure assemblies may provide a general mechanism for off-target drug side effects; 3) the morphoein model of allostery

provides a new structural framework for understanding the physiological control of protein function; and 4) that targeting an oligomer-specific surface cavity on a protein that exists in an equilibrium of non-additive quaternary structure assemblies provides a new approach to allosteric drug discovery. The morphein view of protein quaternary structure dynamics is one of a growing number of exceptions to the one-sequence-one-structure-one-function paradigm, which has dominated our view of protein structure and function. Consideration of a protein as a morphein adds a quaternary structure component to protein structure dynamics in the understanding of disease and in targeting protein structure dynamics in the development of new therapeutics.

ABBREVIATION

PBGS = porphobilinogen synthase

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