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Understanding Microscopic Binding of Human Microsomal Prostaglandin E Synthase-1 (mPGES-1) Trimer with Substrate PGH₂ and Cofactor GSH: Insights from Computational Alanine Scanning and Site-directed Mutagenesis

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Abstract

Microsomal prostaglandin E synthase-1 (mPGES-1) is an essential enzyme involved in a variety of diseases and is the most promising target for the design of next-generation anti-inflammatory drugs. In order to establish a solid structural base, we recently developed a model of mPGES-1 trimer structure by using available crystal structures of both microsomal glutathione transferase-1 (MGST1) and ba3-cytochrome c oxidase as templates. The mPGES-1 trimer model has been used, in the present study, to examine the detailed binding of mPGES-1 trimer with substrate PGH₂ and cofactor GSH. Results obtained from the computational alanine scanning reveal the contribution of each residue at the protein-ligand interaction interface to the binding affinity, and the computational predictions are supported by the data obtained from the corresponding wet experimental tests. We have also compared our mPGES-1 trimer model with other available 3D models, including an alternative homology model and a low-resolution crystal structure, and found that our mPGES-1 trimer model based on the crystal structures of both MGST1 and ba3-cytochrome c oxidase is more reasonable than the other homology model of mPGES-1 trimer constructed by simply using a low-resolution crystal structure of MGST1 trimer alone as a template. The available low-resolution crystal structure of mPGES-1 trimer represents a closed conformation of the enzyme and, thus, is not suitable for studying mPGES-1 binding with ligands. Our mPGES-1 trimer model represents a reasonable open conformation of the enzyme and is, therefore, promising for studying mPGES-1 binding with ligands in future structure-based drug design targeting mPGES-1.

Introduction

Prostaglandins (PG) play a diverse role in many physiological and pathological events in the human body.¹ Prostaglandin E₂ (PGE₂) is the most abundant prostaglandin and exerts its action by interacting with prostaglandin E (EP) receptors-1 to 4.² PGE₂ is a well-characterized mediator of inflammation and pain.³ The biosynthesis of PGE₂ involves conversion of arachidonic acid to PGE₂ and is carried out by three consecutive enzymatic reactions.⁴

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Supporting Information Available. Detailed discussion of the computational and experimental data obtained for the Tyr117Ser, Arg70A/Tyr117Ala, Glu66Ala, Arg67Ala, Glu77Ala, and Thr129Val mutants of mPGEs-1. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Prostaglandin E synthase (PGES) is the terminal enzyme involved in PGE₂ biosynthetic pathway. There are three types of PGES namely microsomal prostaglandin E synthase-1 (mPGES-1), mPGES-2, and cytosolic prostaglandin E synthase (cPGES).⁵ Among these three enzymes, mPGES-1 has been identified as a therapeutically important enzyme. mPGES-1 is an inducible, glutathione dependent, and membrane-bound enzyme.^{6, 7} It has been reported to be induced by proinflammatory stimuli such as interleukins.⁸ mPGES-1 is reported to play a key role in a number of disease conditions, including inflammation, arthritis, fever, pain, cancer, stroke, and bone disorders.⁹⁻¹⁵ Among these, the potential of mPGES-1 as a next-generation anti-inflammatory target has received much attention.¹⁶ The currently available NSAIDs (non-steroidal anti-inflammatory drugs) inhibit either cyclooxygenase (COX)-1 or COX-2 or both.² These inhibitors have been reported to have several deleterious side effects including ulcers, bleeding within the gastrointestinal tract, or increased risk of cardiovascular events.¹⁷ Recent withdrawal of rofecoxib (Vioxx) due to side effects further highlights the need to develop safer anti-inflammatory drugs.² The COX inhibitors prevent the production of all prostaglandins downstream of PGH₂. Blocking the production of prostaglandin-I₂ (PGI₂) has been reported to play a role in cardiovascular events.¹⁸ Unlike COX inhibition, inhibition of terminal mPGES-1 will only block the production of PGE₂ without affecting the production of other prostaglandins including PGI₂. Knock-out studies have identified mPGES-1 as an essential central switch in pyresis.³ mPGES-1 knock-out studies also show a decrease in inflammatory response in a collagen-induced arthritis model.¹⁹ In contrast to COX-2, mPGES-1 deficient mice were reported to be viable, fertile and have normal phenotype.¹⁹ Ischemic stroke induced in mPGES-1 null mice was reported to show significant reduction in the infarct size and volume.^{12, 16} Thus mPGES-1 inhibitors are expected to retain the anti-inflammatory effect as COX inhibitors without the side effects of COX inhibitors.

Recently, several mPGES-1 inhibitors have been identified in experimental screening efforts.¹⁶ Development of new and potent mPGES-1 inhibitors with different scaffolds will help in developing the next-generation of anti-inflammatory agents. To date, there is no three-dimensional (3D) X-ray crystal structure of mPGES-1 in the apo form or with a substrate or inhibitor bound. The absence of an inhibitor-bound mPGES-1 crystal structure is an impeding factor in finding new lead molecules. mPGES-1 is a member of membrane-associated proteins involved in the eicosanoid and glutathione metabolism (MAPEG) family.²⁰ This protein family consists of membrane-bound proteins with diverse functions like leukotriene C₄ synthase (LTCS), 5-lipoxygenase-activating protein (FLAP), microsomal glutathione transferase-1 (MGST-1), MGST-2, and MGST-3.²¹ mPGES-1 shows significant homology with other MAPEG proteins.²¹ There is nearly a 38% amino acid sequence conservation between mPGES-1 and MGST1.²² We have recently reported the first 3D homology model of mPGES-1 trimer and proposed the binding mode of inhibitors and substrates.²³ The computational homology modeling of mPGES-1 structure provided a unique opportunity to explore how mPGES-1 binds with inhibitors and to predict the modes of enzyme-inhibitor binding by carrying out molecular docking. Xing *et al*²⁴ have also reported a homology model of mPGES-1 which is different from our model. Their homology model was built by using a low-resolution crystal structure of MGST1 trimer as a template; the mode of its binding with PGH₂ and GSH was determined by docking and refined by energy minimization only. In contrast, our mPGES-1 model used the protein evolution principle to identify the template structure and the docked mPGES-1-GSH-PGH₂ binding structure was followed by MD simulation in a solvent and membrane environment. Most recently, a crystal structure of mPGES-1 was obtained through electron crystallography by Jegerschold *et al*.²⁵ Naturally, the crystal structure may not necessarily reflect the true structure present in a solvent and membrane environment because of the possible crystal packing and low resolution (3.5 Å in plane) *etc.*, but it should give some valuable insight into the major structural elements.

Generally speaking, computational methods can provide molecular details for both the structural and energetic consequences of mutations, and can address the origin of binding in terms of contributions from electrostatic and van der Waals (vdW) interactions and changes in solvation.²⁶ Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) and Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) approaches have recently been applied to drug discovery for calculating binding affinities of biomolecular complexes, based on molecular dynamics (MD) of the given protein–ligand complex in explicit solvent.^{27, 28} Computational alanine scanning (CAS) involving the MM-PBSA method²⁹⁻³¹ and free energy decomposition involving the MM-GBSA method^{32, 33} have been developed to investigate the binding modes at the atomic level and also to estimate protein stabilities.³⁴ In this work, we address questions regarding the role of selected residues located near the ligand binding sites of the mPGES-1-GSH-PGH₂ complex with regard to catalytic function and substrate binding affinity. As a first step, we analyzed the interaction between the human trimer mPGES-1 structure and PGH₂ and GSH using computational alanine scanning and MM-PBSA free energy decomposition methods to discern key residues. These methodologies proved to be a useful general design tool for analyzing and studying the interactions and stability of the complex, since we have qualitatively estimated the free energy consequences for many mutations from a singular molecular dynamics trajectory. Accordingly, the computed structural and energetic data are in good agreement with available experimental biochemical data and provide valuable insights into the microscopic binding details of mPGES-1 trimer with its PGH₂ substrate and GSH cofactor.

In an effort to gain deeper understanding into the mPGES-1-GSH-PGH₂ recognition mechanism, we studied and compared the relative catalytic rates of the mutants with the wild-type and highlighted the key residues of the mPGES-1 active site involved in the stability of the GSH cofactor. Based on the results of our previous study which highlighted the important conformations of mPGES-1-GSH-PGH₂ and its inhibitor complex, this work may lead to the design of new inhibitors with enhanced affinity and selectivity for mPGES-1. This study may also help to further elucidate the function of this protein family. The objectives of the last part of this study are directed at resolving whether there are significant structural differences in comparison of our developed structural model of the mPGES-1 trimer²³ with the crystal structure and the homology model based on the rat MGST1 trimer structure.²⁴

Materials and methods

Materials

QIAprep Spin Plasmid Kits were purchased from Qiagen, Restriction endonucleases were supplied by New England BioLabs. The pfu polymerase was obtained from Statogene. Oligonucleotide primers were synthesized by MWG Biotech. FreeStyle Max 293 Expression System and 293 free style medium were purchased from Invitrogen. Polyclonal antibodies against mPGES-1 were obtained from the Cayman Chem. Co. PGH₂ and PGE₂ were supplied by Cayman Chem. Co. Other chemicals were from Sigma.

Cloning of mPGES-1

The mPGES-1 cDNA was cloned from human placental cDNA library by PCR techniques. The forward primer is AGCGGATCCATGCCTGCCCACAGCCTG and backward primer is AGAATTCTCACAGGTGGCGGGGCGCT. The mPGES-1 cDNA was cloned into pcDNA3 by inserting PCR product into pcDNA3 vector at BamH1 and EcoR1 sites. The sequence of mPGES-1 in mPGES-1/pcDNA3 construct was confirmed by DNA sequencing.

Preparation of mPGES-1 mutants

Site-directed mutagenesis of mPGES-1 cDNA was performed by the quick change methods as previously described.³⁵ Briefly, the internal primers were designed to contain sense and antisense mutagenic factors with mismatched codons in the wild-type sequence. Pfu DNA polymerase was used for PCR. The products were treated with *DpnI* endonuclease to digest the parental DNA template. All the mutant plasmids were transformed into DH5a cells to amplify plasmid DNA. The DNA sequences of mutants were confirmed by DNA sequencing.

Expression of recombinant mPGES-1 and its mutants in 293-F cells

FreeStyle Max Expression System was used for the expression of wild type and its mutants. Briefly, FreeStyle 293-F cells were cultured following manufacturer's manual in FreeStyle 293 expression medium on orbit rotate shaker in 8% CO₂ incubator at 37°C. Cells were transfected with 1.5 µg/mL of wild type mPGES-1/ pcDNA3 construct or its mutants using FreeStyle Max reagent at a cell density of 1×10^6 for 2 days. Transfected cells were collected, washed and sonicated in TSES buffer (15 mM Tris-HCl, pH 8.0 plus 0.25 M sucrose, 0.1 mM EDTA and 1mM DTT) on ice. The broken cells were first centrifuged at $12,500 \times g$ for 10 min. The supernatant was further centrifuged at $105,000 \times g$ for 1 hr at 4°C. The pellet was washed and homogenized in PBS buffer. The crude microsomal mPGES-1 preparation was aliquoted and stored at -80°C. The crude protein concentration was 8 mg/mL.

mPGES-1 enzyme activity assay

The enzyme activity was assayed as described previously with some modifications.³⁵ Briefly, the reaction mixture contained: 0.2 M Na₂HPO₄/NaH₂PO₄, pH 7.2, 10 µL; 0.1 M GSH, 2.5 µL; diluted microsomal enzyme (80 µg/mL), 1-3 µL; PGH₂ (0.31 mM in DMF), 5 µL, and H₂O in a final volume of 100 µL. PGH₂ was stored in dry ice and used to initiate the reaction. The reaction was carried out at room temperature for 2 to 3 min. Ten µL of SnCl₂ (40 mg/mL) in ethanol was then added to stop the reaction. The non-enzymatic conversion of PGH₂ to PGE₂ was performed in the same buffer devoid of enzyme. The reaction mixture was placed on ice until PGE₂ production was determined by PGE₂ enzyme immunoassay as described earlier.³⁶

Computational alanine scanning

The computational alanine scanning (CAS) method was used to estimate the relative binding affinity of different mPGES-1 mutants to the GSH cofactor and PGH₂ substrate. The alanine mutant structures were generated by altering the coordinates of the wild-type trajectory obtained from our previous MD simulation.²³ This method involved deleting atoms and truncating the mutated residue at C_γ by replacement with a hydrogen atom.³¹ All parameters in the topology files for the mutated residues were replaced by the alanine residue parameters. For the binding free energy calculations, a total of 200 snapshots of the complexes were extracted from the last 400 ps of the MD trajectory with a 2 ps time interval and subjected to energy minimization (see below). The same 200 snapshots were also used to calculate the per-residue decomposition energies. The binding free energy difference between the mutant and the wild type complexes is defined as:

$$\Delta\Delta G_{\text{bind}} = \Delta G_{\text{bindmutant}} - \Delta G_{\text{bindwild type}}$$

Energy minimization and MD simulations in implicit solvent model

The energy minimization and short MD simulations (100 ps) were performed by using the Sander module of Amber9 program.³⁷ During the simulations, only the mutated residue side chain and ligands (PGH₂ and GSH) were allowed to move. The nonbonded interaction cutoff

and dielectric constant were set up to group-based (20-Å cutoff distance) and distance-dependent ($\epsilon = 4r$)^{38, 39} to mimic the solvent environment, respectively. The MD simulation was performed with a time step of 1 fs. The final desired temperature of 298 K was obtained by requesting a heating cycle from 0 to 298 K over the course of the first 5000 MD steps and the Langevin dynamics was used to simulate solvent frictional effects with a collision frequency $\gamma = 1.0 \text{ ps}^{-1}$.

Structural modeling of the mPGES-1^{Thr129Val} and mPGES-1^{Tyr117Ser} mutants

To maintain consistency with the method used to compute the relative binding free energies upon the mutations, the side chain and the relative binding free energies of the Thr129Val and Tyr117Ser mutants were also calculated by the CAS method. The starting coordinates of these mutant structures were generated by altering the coordinates of the 200 snapshots of the wild-type trajectory. Then, the final structures were also energy-minimized by using Amber9 program.³⁷

MM-PBSA/MM-GBSA calculation

The binding free energies were calculated using the MM-PBSA/MM-GBSA method implemented in Amber9 program.^{23, 40, 41} Our MM-PBSA calculation for each snapshot (wild-type and mutants) was carried out in the same way as in other protein-ligand systems.²³ Briefly, the MM-PBSA method can be conceptually summarized by the following equations:

$$\Delta G_{\text{bind}} = \Delta G_{\text{complex}} - \Delta G_{\text{protein}} - \Delta G_{\text{lig}} \quad (1)$$

$$G = E_{\text{gas}} + G_{\text{sol}} - TS \quad (2)$$

$$E_{\text{gas}} = E_{\text{int}} = E_{\text{vdw}} + E_{\text{ele}} \quad (3)$$

$$G_{\text{sol}} = G_{\text{PB}} + G_{\text{np}} \quad (4)$$

$\Delta G_{\text{complex}}$, $\Delta G_{\text{protein}}$, and ΔG_{lig} are the free energy changes of the complex, protein, and ligand, respectively. Each free energy change from Eq. (1) was calculated by summing the gas phase internal energy (E_{gas}), the solvation free energy (G_{sol}), and the entropy term ($-TS$) in Eq. (2). E_{gas} is the standard force field energy, including the internal energy as well as the non-covalent van der Waals and electrostatic energies (Eq. (3)). The solvation free energy, G_{sol} , was calculated using the PB/SA model, which decomposes the solvation free energy into the sum of the electrostatic component (G_{PB}) and non-polar component (G_{np}) (Eq. (4)). The electrostatic component was calculated using the PBSA program with the default cavity radii from the Amber9 file. The dielectric constant was set to 1 for the interior solute and 80 for the surrounding solvent. The LCPO method⁴² was used to calculate the Solvent Accessible Surface Area (SASA) for the estimation of the non-polar solvation free energy (ΔG_{np}) using Eq. (5) with $\gamma = 0.00542 \text{ kcal/mol}\text{\AA}^{-2}$ and $\beta = 0.92 \text{ kcal/mol}$.⁴³

$$\Delta G_{\text{np}} = \gamma \text{SASA} + \beta \quad (5)$$

For the MM-GBSA method, G_{PB} is replaced by G_{GB} . Hawkins, Cramer, and Truhlar's pairwise, generalized Born model^{44, 45} was used with parameters described by Tsui and Case. The LCPO method was used to calculate the SASA with $\gamma = 0.005 \text{ kcal/mol}\text{\AA}^{-2}$ and $\beta = 0.00 \text{ kcal/mol}$.³⁸

We employed a single molecular dynamics trajectory protocol, which can qualitatively estimate the free energy consequences of many mutations.³¹ As a consequence, the contribution of internal energy to the binding energy is equal to zero. Since the calculation of the entropic contribution ($-T\Delta S$) requires several approximations and provides only a rough estimate, especially in the case of a simulation in which only a small portion of the protein moves, we calculated only the binding energy. As previously demonstrated by Massova and Kollman,³¹ the entropic contribution cancels out when the wild-type and mutants bind to the same receptor.

Results and Discussion

Selection of residues for mutagenesis

The human isoenzyme mPGES-1 has been the subject of intensive research because of its ability to detoxify a wide range of xenobiotics. It is recognized as a member of a group of membrane associated proteins in MAPEG family.²⁰ On the basis of our previous model,²³ the initial interaction between the PGH₂ and mPGES-1-GSH complex may occur in the binding pocket defined by two subunits of the mPGES-1 trimer. Hence, we have shown that the carboxylate group of PGH₂ substrate interacts with the mPGES-1-GSH complex by establishing a strong salt bridge with the highly conserved Arg110 residue and a weak hydrogen bond with the hydroxyl group of Thr129. While the scaffold of GSH cofactor is maintained in a quasi-extended conformation by interacting with the α -helix TM2 (residues 62 to 92) of the mPGES-1. In order to provide a clear picture of the positions of the mPGES-1 key residues interacting with the GSH and PGH₂, Figure 1 depicts the two orientations of the mPGES-1-GSH-PGH₂ binding pocket. Hence, the GSH sub-pocket, P^{GSH} , is defined by the residues Arg70, Asn74, Arg73, Glu77, Tyr117, Leu121, Arg122, and Arg73' while the PGH₂ sub-pocket, P^{PGH_2} , is defined by the residues Arg126, Thr129, Arg110, His72', Lys26', Leu69', Ile125, Asn74, and Arg73' at the interface of the two mPGES-1 subunits.

In light of these earlier results, we investigated both residue mutation experiments and *in silico* studies to obtain deeper insights into the positioning of GSH and PGH₂ in the mPGES-1 trimer. In particular, Tyr117 was mutated to alanine and serine, Thr129 was mutated to valine, and Arg70 and Arg122 were simultaneously mutated to the double mutant Arg70Ala/Arg122Ala. The mutations of these key residues are believed to help not only explain the factors affecting the binding mode of PGH₂ substrate and/or GSH cofactor to the mPGES-1 trimer, but also assess their contributions to the substrate binding and catalysis. The mutagenesis results in this study are not meant to reproduce the precise energies of the corresponding mutations as measured experimentally, but to provide an energetic estimate of the interactions between the ligands (GSH and PGH₂) and the residues mutated in the complex of the wild-type mPGES-1-GSH-PGH₂.

Effect of point mutations on $\Delta\Delta G_{\text{bind}}$ for PGH₂ in the mPGES-1-GSH complex

Computational alanine scanning (CAS) was undertaken to elucidate the key residues in the binding site of the mPGES-1 enzyme and to gain further insight into the contributions of the various energy terms to the binding free energy differences, the change in the van der Waals and electrostatic interaction energies, as well as the electrostatic and nonpolar desolvation free energies upon mutation. This method is dependent on the assumption that local changes in the protein do not significantly influence the overall conformation of the complex. The relative binding free energies calculated by CAS, using both MM-PBSA (PB) and MM-GBSA (GB)

methods, for the R70A, N74A, Y117A, R122A, and R70A/R122A mutants, are given in lines 9 and 14 of Table 1. The associated experimental values are given in line 16 of the same Table and Figure 2. $\Delta\Delta G_{\text{bind}}$ can be viewed as an indicator of the importance of the side chain on the stability of the GSH and PGH₂ ligands in mPGES-1 structure. The negative and positive values of $\Delta\Delta G_{\text{bind}}$ ($\Delta G_{\text{mutant}} - \Delta G_{\text{wild-type}}$) indicate favorable and unfavorable outcomes, respectively. In both the GB and PB methods, the calculated relative binding free energies are positive, illustrating that the PGH₂ binding is slightly weaker with all mutants compared to the wild-type mPGES-1 enzyme. This conclusion is in agreement with experimental findings and illustrates that positive values in our calculations correlate to the experimentally unfavorable alanine substitutions for mPGES-1 structure.

Larger relative free energy deviations were obtained by the PB than by GB model. Thus, except for N74A mutant (detailed discussion is given in the next section), the calculated relative binding free energies exhibit an average deviation of ~ 0.2 kcal/mol from experiment according to the GB model, whereas the average deviation increases to ~ 1.0 kcal/mol from experiment according to the PB model. We note that the PB model is more sensitive to the atomic coordinates than the GB model during the calculation of the solvation energy contribution.

In addition to our mutation experiments discussed above, we computed the relative binding free energies of several mutants (E66A, R67A, H72A', E77A, and R70A/Y117A) that were successfully predicted to be important for the catalytic activity of the mPGES-1 enzyme.²⁵ As shown in Table 1, the present CAS calculations revealed these mutations also cause small changes in the relative binding free energies of PGH₂ to the enzyme. Indeed, all of these mutants, except H72A', all lie on one side of the binding pocket defined by the TM2 and TM3 α -helices, which we contend to have an influence on the GSH fold and contribute significantly to the binding of the cofactor to mPGES-1.

To gain further insight into the contributions of each component to the binding free energy changes, we computed the change in the intermolecular vdW and electrostatic interactions and the polar and nonpolar solvation free energies upon alanine mutation in Table 1. In most cases the change of intermolecular electrostatic interactions is seen to strongly anticorrelate with the change of polar solvation free energy. For example, the intermolecular electrostatic interactions ($\Delta\Delta E_{\text{ele}}$) between the positively charged R70, R122, and R67 of mPGES-1 and the negatively charged PGH₂ are strongly favored in the wild-type complex, but less desolvation penalties ($\Delta E_{\text{sol}}^{\text{GB}}$) are paid in the mutants. Conversely, the intermolecular electrostatic interactions ($\Delta\Delta E_{\text{ele}}$) between the negatively charged E66 and E77 residues of mPGES-1 and PGH₂ are less favorable in the wild-type complex, but larger polar solvation free energies ($\Delta E_{\text{sol}}^{\text{GB}}$) are unfavorable for these mutant residues. One exception is the N74A mutant which will be discussed below.

Effect of point mutations on $\Delta\Delta G_{\text{bind}}$ for GSH in the mPGES-1 enzyme

In order to explore the relative enzymatic activity of the mPGES-1 mutants, we also performed Computational Alanine Scanning (CAS) study on GSH cofactor binding to the wild-type and above-mentioned mPGES-1 mutants. The relative catalytic activities $k_{\text{cat}}^{\text{Mut}}/k_{\text{cat}}^{\text{WT}}$ of the enzyme mutants were determined by steady-state kinetic analysis and the results are listed in Table 2 and Figure 2. The CAS results for relative binding affinities are also summarized in Table 2 with respect to the wild-type mPGES-1. Below, we analyze in detail these mPGES-1 mutants and the recently published mutants.²⁵

Arg70Ala mutant

Arg70 was found computationally to be important for the stability of the head-tail of GSH cofactor in the mPGES-1 active site.²³ GSH is bound in a quasi-extended conformation to the

side of the TM2 α -helix by forming several hydrogen bonds to the residue side chain. As illustrated in our mPGES-1-GSH-PGH₂ complex model, GSH is associated with a subunit through electrostatic interactions between the γ -glutamyl carboxyl group of the ligand and the side chains of Arg70 and Arg122 (Fig. 1). The Arg70Ala mutation is curiously found experimentally to decrease only slightly the catalytic activity (~60%) of the enzyme (based on our biochemical data and *ref.*²⁵). In order to understand the modest effects of the Arg70Ala mutation, we carried out a short MD simulation (100 ps) to relax the mutated residue and the ligands. The simulated mPGES-1^{R70A}-GSH-PGH₂ structure showed a minor motion of the GSH γ -glutamyl carboxyl tail but still maintains the GSH scaffold in the same binding mode, by interacting with the neighboring Arg122 side chain. We only carried out the MD simulation for 100 ps because the MD-simulated protein-ligand binding structure was stabilized quickly after the initial motion within the first 10 ps. This small conformational change resulted in a correspondingly small variation in the calculated relative binding free energy $\Delta\Delta G_{\text{bind}}^{\text{GSH}}$ (~0.8 kcal/mol) of GSH upon alanine mutation.

Asn74Ala mutant

Although the experimental relative binding free energy of PGH₂ upon N74A mutation showed a small variation of 0.46 kcal/mol, it is found computationally to be important in the GSH and PGH₂ affinities to the wild-type mPGES-1 and corresponded to a large calculated $\Delta\Delta G_{\text{bind}}^{\text{PGH}_2}$ values of ~5.6 (PB model) and ~4.7 kcal/mol (GB model), respectively. Our overestimate of the $\Delta\Delta G_{\text{bind}}$ for the polar N74 residue involves buried salt bridges/hydrogen bonds and suggests that our assumption of minimal conformational change upon the mutation is less justified in this case. However, this overestimate can be explained structurally. The large excess in calculated relative free energies of binding can be explained by destabilization of the GSH cofactor that may occur upon N74A mutation. As mentioned previously,²³ residue Asn74 forms hydrogen bonds with polar and charged carboxylate tail groups of GSH, and these intermolecular forces are reflected by their significant contribution to electrostatic interactions in the binding free energy (see Table 2). The N74A mutation eliminates the important hydrogen bonds between the GSH and receptor, which cannot be compensated for without a significant structural reorientation of GSH and PGH₂ which are in close contact. In addition to this structural perturbation, the overestimation of the relative binding free energy for the N74A mutant may also be attributed to the fact that the CAS method employed in this study cannot accurately account for the effects of a significant change of the protein-ligand binding mode upon the mutation. The change from the side chain of the polar residue to a smaller, hydrophobic alanine side chain could induce a significant change of the protein-ligand binding mode in the mPGES-1 active site.

Compared to the wild-type complex, the intermolecular interactions ($\Delta\Delta G_{\text{GAS}}$) of both PGH₂ to the mPGES-1^{N74A}-GSH and GSH to the mPGES-1^{N74A} become weaker. Accordingly, the difference in van der Waal interaction (ΔE_{vdW}) for the PGH₂ interaction is also smaller in the mutated protein. To our surprise, in this mutant the relative electrostatic desolvation penalty is ~2 kcal/mol (using the PB model) and ~1 kcal/mol (using the GB model), respectively (Table 1). This penalty is due to the loss of a strong electrostatic interaction from Asn74 and cannot be completely compensated for by the increase of the desolvation penalty due to the hydrophobic residue mutant. However, the inclusion of change in entropy upon mutation ($-T\Delta S$) should bring the calculated relative binding free energy $\Delta\Delta G_{\text{bind}}^{\text{PGH}_2}$ closer to the experimental value.

Similar results are observed for the GSH interaction. Indeed, the conformational change of both GSH and PGH₂ due to the N74A mutation reduces the binding affinity of these ligands to the mutated protein. This change may also account for the mutation in this residue giving

rise to a relatively large deviation in $\Delta\Delta G_{\text{bind}}^{\text{GSH}}$ (~4.2 kcal/mol), and leading to nearly a complete inhibition of the catalytic activity (16%) of the enzyme (Tables 1 & 2).

Arg122Ala mutant

Similar to what described above for the R70A case, a short MD-simulated (~100 ps) mPGES-1^{R122A}-GH-PGH₂ complex structure induced a minor conformational change in the GSH scaffold leading to a specific γ -glutamyl carboxyl interaction and an orientational change toward the Arg70 side chain, yet without affecting the PGH₂ binding mode ($\Delta\Delta G_{\text{bind}}^{\text{PGH}_2} = \sim 0.3$ kcal/mol). Thus, we observe in Figure 1 that when one arginine is mutated to alanine the other neighboring arginine may hold the GSH in its initial position. This result is confirmed by the resulting lower relative binding free energy of GSH in these mutants obtained by CAS calculations (Table 2). The calculated relative binding free energies of GSH in the R70A and R122A mutants with respect to the wild-type mPGES-1 revealed $\Delta\Delta E_{\text{elec}}$ interaction decreased by ~54 and ~24 kcal/mol for the R70A and R122A mutants, respectively. By adding the electrostatic solvation penalty term, we observed that the R70A mutant is reduced approximately two-fold compared to the relative binding free energy of the R122A mutant. Also, this is consistent with the experimental data reflecting a higher catalytic activity for the R122A *versus* the Arg70A mutant.

Arg70Ala/Arg122Ala mutant

To gain better insight into the interaction between the GSH and the two arginine residues (70 and 122), we tested experimentally the catalytic activity of the double mutant R70A/R122A and compared the result to the CAS study. The relative binding free-energy analyses showed that the double mutant of the mPGES-1 significantly reduced the binding affinity of GSH ($\Delta\Delta G_{\text{bind}}^{\text{GSH}} = \sim 2.48$ kcal/mol) in the mutated enzyme, whereas that mutant has little effect on the PGH₂ binding mode. Thus, the conformational changes of the GSH binding mode due to the loss of the sandwich interaction between the GSH γ -glutamyl carboxyl group, Arg70 and Arg122 side chains upon alanine mutation may explain the reduced affinity of the GSH cofactor to the mPGES-1 double mutant. In agreement with our computational study, we observed that the experimental relative catalytic activity of the mutated protein decreased dramatically (24%).

Tyr117Ala mutant

Since Tyr117 residue has been suggested to play an important role in the catalytic activity of the MAPEG protein,²⁰ we focused attention on this residue. Tyr117 is located in the *P*^{GSH} active site near the catalytic site and, based on our previous docking study²³, this residue may be involved in the enzymatic activity by stabilizing and properly orienting the nucleophilic thiolate group through a dipole-quadrupole interaction. Hence, this residue may assist in the catalysis of the PGH₂ epoxy group.

In our 3D mPGES-1-GSH-PGH₂ model, the βCH_2 group of the GSH L-cysteinyglycine moiety is stabilized by a dipole-quadrupole interaction with the Tyr117 aromatic ring, while the cysteinyl backbone of GSH is involved in hydrogen bonding with Asn74. The phenyl ring side chain of this residue adds a favorable 2.73 kcal/mol contribution to the binding free energy, derived mostly from the vdW contact ($\Delta\Delta E_{\text{vdW}} = \sim 1.8$ kcal/mol) with GSH (Table 2).

Interestingly, it was found that when Tyr117 is mutated to Phe, the mPGES-1 still maintains full catalytic activity relative to the wild-type.²⁵ Indeed, during our previous MD simulation of the mPGES-1-GSH-PGH₂, it was not possible to find any hydrogen bonding between the Tyr117 hydroxyl group and the GSH, which may explain the insignificant affect of $\Delta\Delta E_{\text{elec}}$ upon Y117A mutation. It appears that the aromatic ring of Tyr117 plays an important role in maintaining the function of mPGES-1, which was hinted by the free energy decomposition

results below. These results show a nearly double contribution from the vdW contact than from the electrostatic interaction. This result is also in reasonable agreement with the decreased relative free energy of GSH ($\Delta\Delta G_{\text{bind}} = \sim 2.47$ kcal/mol) and the large loss of the relative catalytic activity (24%) upon mutation.

His72'Ala mutant

The experimental results have shown that mutation of this residue to alanine markedly decreases the mPGES-1 activity (32%). Indeed, this finding and the large $\Delta\Delta G_{\text{bind}}^{\text{PGH}_2}$ value obtained from the CAS calculation may be interpreted on the basis of the structural model of mPGES-1-GSH-PGH₂ complex (Table 1 & 2). His72' residue is located on the TM2 α -helix of the second mPGES-1 subunit and is involved in a π - π aromatic interaction with the ω -chain group of the PGH₂ substrate. In the same segment there is also located the Lys26' residue, which form cation- π interaction with His72' (Fig. 1 & 2). The His72A' mutation may have two independent consequences: (1) the loss of a π - π aromatic interaction of the histidine side chain with the double bond of the PGH₂ ω -chain and (2) the introduction of a cavity. The cavity could either be filled by water molecules or by a rearrangement of the PGH₂ ω -chain. Thus, the loss of this interaction and the rearrangement of the PGH₂ ω -chain could affect the binding mode of PGH₂ to the Arg110 side chain and the GSH conformation which is in close contact with the PGH₂ substrate. In addition, His72' is involved in direct amino-aromatic interaction with Lys26'. Amino-aromatic interaction has been found in several proteins to have certain structural and mechanistic implications such as an increase in the stabilization energy and an elevation of the Histidine pK_a value.^{47, 48} In total, these consequences may affect PGH₂ and GSH binding and result in reduced affinity (increased K_M) of the enzyme for both ligands, as computed by the CAS method (Tables 1 and 2).

In addition to the mutants discussed above, we also carried out combined computational and experimental studies on other mutants of mPGES-1 including the Tyr117Ser, Arg70A/Tyr117Ala, Glu66Ala, Arg67Ala, Glu77Ala, and Thr129Val mutants, demonstrating how these amino acid residues also affect the catalytic activity of the enzyme. Detailed discussion of the computational and experimental data obtained for these mutants are provided in Supporting Information. Finally, although the computational alanine-scanning mutagenesis method can conveniently predict relative binding affinities ($\Delta\Delta G_{\text{bind}}$ values), for some special systems (N74A and His72' mutants), such mutations induce large conformational changes, and thus entropic contributions need to be accounted for in binding free energy calculations.

Decomposition of binding energy on a per-residue basis

Energy decomposition is used to probe which residues generate significant intermolecular contributions to the binding ligands (GSH and PGH₂) and serves as an easier alternative to computational alanine scanning mutagenesis. The per-atom contributions can be summed over atomic groups such as residues, backbones, and side chains, to obtain their contributions to the total binding free energy.⁴⁹ Thus, contributions due to "non-mutable" functional groups have been calculated at the atomic level using the MM-GBSA method.⁵⁰ Table 3 reports the decomposition of the binding energy into contributions from vdW energy, electrostatic interaction energy and solvation free energy, for PGH₂, GSH, mPGES-1-GSH and mPGES-1 individually. Table 4 shows the decomposition of $\Delta G_{\text{gas+solv}}$ on a per residue basis for mPGES-1.

The total binding energies of GSH and PGH₂ to the mPGES-1 (or mPGES-1-GSH for the PGH₂) are -19.6 and -62.4 kcal/mol (Table 3), respectively. These values are ~ 0.2 kcal/mol higher than the values -19.84 and -62.34 kcal/mol calculated via the MM-PBSA method (Tables 1 & 2). Here, the GSH cofactor and mPGES-1 trimer contribute -1.6 and -18.0 kcal/

mol each. In contrast, PGH₂ substrate contributes about ~42 % (-26.4 kcal/mol) to the total binding energy (-62.4 kcal/mol) of the mPGES-1-GSH-PGH₂ complex.

For the PGH₂ substrate, the binding energy sum (-4.5 and -27.5 kcal/mol, respectively) from the *P*^{GSH} and *P*^{PGH₂} sub-pocket residues comprises 12% and 76% of the entire mPGES-1-GSH binding energy (-36.0 kcal/mol), and the remaining 12% of the binding energy is attributed to the residues outside the binding cavities.

Arg110 residue contributes -11.9 kcal/mol primarily from the guanidinium side chain and essentially through electrostatic interactions. (Table 4). His72' has -4.4 kcal/mol contribution to the PGH₂ binding energy whereas it has 4.77 kcal/mol contribution ($\Delta\Delta G_{\text{bind}}^{\text{GB}}$) and 5.01 kcal/mol contribution ($\Delta\Delta G_{\text{bind}}^{\text{PB}}$) to the binding energy after being mutated to alanine (Table 1). Including the approximations in these models and the dependency upon parameters, the agreement is encouraging. Interestingly, the decomposition of the binding energy shows that Arg110 contributes about 33 % (-11.9 kcal/mol) to the total binding energy (-36.0 kcal/mol) of the PGH₂ to mPGES-1-GSH complex, highlighting the specific interaction of PGH₂ to Arg110. Whereas Asn74 contributes only about -1.3 kcal/mol leading to the conclusion that the side chain of this residue has a small effect on the PGH₂ binding, as observed experimentally. In addition, the Table 4 shows that except for Arg110 and His72', the residues of both *P*^{GSH} and *P*^{PGH₂} sub-pockets contribute minimally to the PGH₂ binding energy, which is in excellent agreement with the experimental data showing a small variation in the $\Delta\Delta G_{\text{bind}}^{\text{PGH}_2}$ upon the alanine mutation.

Surprisingly, the decomposition of the binding energy shows that Arg126 contributes -2.1 kcal/mol to the PGH₂ binding energy, mainly due to the long-range electrostatic interaction since its guanidinium side chain is distant to the PGH₂ endoperoxide O–O bond by only ~3.7 Å. This result is very reasonable because it is in excellent agreement with the recent biochemical data showing that the R126A and R126Q mutants strongly reduce the isomerase activity of mPGES-1.⁵¹

In the case of GSH cofactor, the summed binding energies (-16.2 and -1.5 kcal/mol, respectively) from the *P*^{GSH} and *P*^{PGH₂} sub-pocket residues occupies about ~90% and ~8% of the full mPGES-1 binding energy (about -18.0 kcal/mol). From the analysis of Table 4 we contend that the decomposition of the binding energy of Arg70, Arg73, Asn74, and Arg73' residues showed the largest contributions, suggesting that they play an important role in cofactor binding due primarily to the electrostatic ΔE_{elec} energy term.

Arg73 forms hydrogen bonds with the GSH carboxylate group and contributes about -5.1 kcal/mol to the binding of GSH, which are reflected by the major contribution of electrostatic interactions to the binding energy (ΔE_{ele} term, see Table 4).

More importantly, the results of the energy decomposition show that most of the contribution of Tyr117 for the binding comes from van der Waals interactions. This is in excellent agreement with the experimental data illustrating that the Y117F mutation does not change the catalytic activity of the mPGES-1 enzyme. Also, this confirms our CAS study of the interaction between the cysteinyl group of GSH and the Tyr117 phenyl ring. Indeed, the behavior of Tyr117 aromatic ring seems to play a role in holding the extended GSH conformation, since the Tyr117 mutation to a smaller residue (Ala or Ser) disrupted this van der Waals contact leading the GSH thiol group to move in the engendered cavity. The structural perturbation of GSH due to this mutation is confirmed by the biological data, demonstrating that the mutant had no effect on the PGH₂ binding mode but gives rise to a lower catalytic activity (24%) of the mutated enzyme.

Arg73 and Arg73' residues which are not mutated to alanine in the CAS method are recognized as critical residues (Figures 1 & 3). In this case, the contributions of Arg73 and Arg73' arise

more from the side chains than the backbone atoms (Table 4). Except for van der Waals contacts, electrostatic interactions from these residue side chains contribute substantially to the binding free energy (Table 4). Arg73 contributes about -5.1 kcal/mol to the GSH binding affinity, more than ~70% of which originates from the guanidinium side chain according to the free energy decomposition calculation. Interestingly, Arg73 also appears to favor the PGH₂ binding, but to a lesser extent.

Correlation of relative rate constants $k_{cat}^{Mut}/k_{cat}^{WT}$ of mPGES-1 with $\Delta\Delta G_{bind}^{GSH}$

All studies were highlighted by a significant decrease in ΔG_{bind} for GSH upon mutation. Individually, the $\Delta\Delta G_{bind}^{GSH}$ values calculated for the Y117A, Y117S, R70A, and R70A/R122A mutants are all lower than the wild-type by 2.47, 5.11, 0.8, and 2.48 kcal/mol, respectively. Conversely, no significant differences were found in $\Delta\Delta G_{bind}$ for PGH₂ substrate when compared to the wild-type enzyme. It appears that the reduction in catalytic activity of these mutants is not due to a decrease in $K_M^{PGH_2}$ but rather to a decrease in the binding between mPGES-1 with GSH (denoted by K_d , not measured experimentally). This observation is in reasonable agreement with the CAS study and the decomposition of the binding energy of this complex suggests that the mutated residues exhibit an effect on the mode of the GSH binding.

As described above, the residues of the *P* PGH₂ sub-pocket (Table 4) which were not mutated comprise about 64% of the total binding energy of PGH₂ to the mPGES-1-GSH complex. The binding energy decomposition (Table 4) shows that the mutated residues (this study and previous data) contribute slightly to the total binding energy of PGH₂ to mPGES-1-GSH complex. On the other hand, except for Arg110 and Thr129 residues which are involved in the PGH₂ interaction with the enzyme, the binding energy decomposition relative to GSH shows larger values for the mutants in this study. These data suggest that modification of one or more residue(s) at, or close to, the GSH sub-pocket is required to inhibit the mPGES-1 enzyme. The effects of these mutations are compatible with the important structural role of both PGH₂ and GSH ligands binding to mPGES-1. However, the N74A and Y117S mutants showed a substantially lower affinity (greater K_d) toward GSH, indicating that the impact of these positions upon binding with GSH is probably through hydrogen bonding and/or active-site rearrangement.

We examined the possible correlation between the catalytic activity of the enzyme and its affinity of binding with GSH cofactor. As the catalytic rate constant (k_{cat}) of an enzymatic reaction is determined by the activation free energy (ΔG_{av}) of the reaction, the ratio of the k_{cat} value for a mutant to the k_{cat} value for the wild-type is determined by the mutation-caused shift of the activation free energy ($\Delta\Delta G_{av}$):

$$\Delta\Delta G_{av} = -RT \ln \left(\frac{k_{cat}^{Mut}}{k_{cat}^{WT}} \right) \quad (6)$$

Assuming that $\Delta\Delta G_{av}$ linearly correlates with the mutation-caused shift of the binding free energy ($\Delta\Delta G_{bind}^{GSH}$) for mPGES-1 with GSH, we may obtain the following empirical correlation relationship:

$$\Delta\Delta G_{bind}^{GSH} \approx w \left[-RT \ln \left(\frac{k_{cat}^{Mut}}{k_{cat}^{WT}} \right) \right] + \text{constant} \quad (7)$$

in which w is a proportional constant. To investigate the relevance of calculated relative binding free energy ($\Delta\Delta G_{bind}^{GSH}$) to the experimental relative rate constant (relative catalytic activity) of

the studied mutants, we first plotted the relative binding free energy $\Delta\Delta G_{blind}^{GSH}$ versus the $(k_{cat}^{Mut}/k_{cat}^{WT})$ for all mPGES-1 mutants (Figure 4). The relative rate constant in this study is taken as the relative k_{cat} value of the mPGES-1, i.e. $k_{cat}^{Mut}/k_{cat}^{WT}$. It is evident that the CAS approach is successful at achieving the correct relative rankings and there is a high linear correlation ($R = 0.967$) observed between the calculated $\Delta\Delta G_{blind}^{GSH}$ and experimental $k_{cat}^{Mut}/k_{cat}^{WT}$ values. The lack of a significant correlation for the R67A and E77A mutants may indicate that structural parameters such as the positions and/or conformations of the residues are not directly involved in the rate-determining step. These results further support our mPGES-1 model as these residues (R67 and E77) contribute primarily to the stability of the complex with the mPGES-1 trimer.

Whereas the absence of a perfect correlation might be expected given the complexity of factors determining $\Delta G^\ddagger$, k_{cat} , and ΔG_{bind} , it is gratifying that $\Delta\Delta G_{blind}^{GSH}$ for the R70A/R122A double mutant and the R70A and R122A single mutants are highly correlated. These findings are consistent with the important role assigned to the Arg70 and R122 side chains for the GSH binding in the pocket as revealed by our structural, kinetic, and computational studies.

We also plotted the relative binding free energy $\Delta\Delta G_{blind}^{GSH}$ versus the $\ln(k_{cat}^{Mut}/k_{cat}^{WT})$ for all mPGES-1 mutants, except for R67A and E77A (Figure 4 & eqn. 7). Here, it is also interesting to note the high linear correlation ($R = 0.945$). Indeed, if the mutation sites are accompanied by extensive structural changes in the enzyme or the Michaelis-Menten complex, the free energy barrier change calculated for one mutation (R122A) may well depend on the presence of other mutations (R70A).

The empirical equation (eqn. 6) discussed here suggests an interesting correlation between the mutation-caused changes of the catalytic activity of mPGES-1 against substrate PGH_2 and the corresponding shifts of the mPGES-1-GSH binding free energy produced by the mutations on the residues nearby GSH. The correlation highlights the importance of the binding of cofactor GSH with the mPGES-1 trimer in maintaining the catalytic activity of the enzyme. When the binding of GSH with mPGES-1 becomes weaker due to an amino acid mutation, the binding of GSH with the mPGES-1 mutant could slightly change from the ideal orientation of GSH in the active site of the wild-type enzyme. A minor change from the ideal orientation of GSH in the active site could decrease the catalytic activity. The lower the binding affinity between GSH and an mPGES-1 mutant, the larger the change from the ideal orientation could be. A larger change from the ideal orientation of GSH in the active site could lead to a larger decrease in the catalytic activity of the enzyme.

Homology modeling of mPGES-1 trimer based on the MGST-1 trimer template

It is interesting to compare our 3D model of mPGES-1 trimer with the homology model reported by Xing *et al.*²⁴ and the crystal structure (3dww.pdb). In our previous work,²³ we first built the 3D model of mPGES-1 monomer by performing homology modeling using the rat MGST1 structure as a template for the monomer. Starting from the built mPGES-1 monomer structure, we decided to use the relative positions and symmetry of the 12 α -helices in the homologue ba3-cytochrome c oxidase structure as a template to develop the full homotrimer model since it was found that the mPGES-1, MGST1, and the cytochrome c oxidase have a common evolutionary origin^{46, 52} and their homologous relationship may include three-dimensional similarities.^{53, 54} The homologue ba3-cytochrome c oxidase structure was used to determine the relative positions of the three equivalent mPGES-1 subunits in the mPGES-1 homotrimer. Each subunit of the homotrimer has the same 3D structure of the mPGES-1 monomer. We used the ba3-cytochrome c oxidase structure, instead of the MGST1 trimer structure, as a template to build the 3D model of mPGES-1 trimer because of the low level of

sequence identity (~39%) between mPGES-1 and MGST1. Since in the present case, we do not expect the mPGES-1 trimer to have the identical structural packing environment found in the MGST1 trimer form.

According to our MD-simulated mPGES-1 trimer structure²³ and a visual inspection of the recently published crystal structure, each subunit (monomer) has a TM helix (residues 62 to 92) facing toward the center of the trimer giving rise to the formation of a strong hydrophobic core (Figure 5). The interactions within the core are characterized mainly by a network of dipole–quadrupole interactions between the phenyl ring of Tyr80, Phe84, Phe87, and Phe91 of one subunit and the equivalent residues of the other two subunits. In addition, the hydrophobic core of the trimer is stabilized by the hydrogen bonding of the hydroxyl groups of Tyr80, Tyr80', and Tyr80'' with the backbone oxygen atoms of the TM helices facing the core. As Xing *et al.*²⁴ did not describe such type of interactions in their homology model, we decided to also build another mPGES-1 trimer model (denoted by mPGES-1^(MGST1)) using the rat MGST1 trimer structure as a template for comparison between the two trimer models. Model mPGES-1^(MGST1) is expected to be similar to that built by Xing *et al.*²⁴ Depicted in Figure 5 is the structural information about the mPGES-1^(MGST1) homotrimer. A visual inspection of the mPGES-1^(MGST1) homotrimer structure reveals that the core structure of the trimer is too compact to make favorable interactions between the three equivalent subunits of the trimer. Even after energy-minimization and MD simulation of the mPGES-1^(MGST1) homotrimer side chains, residues Y80, F84, F87, and F91 still exhibited steric clashes with the equivalent residues of the other two subunits. This finding is enhanced by the sequence alignment between the human mPGES-1 and the rat MGST1 proteins.²³ The residues V83, G87, L90, and L94 of the MGST1 structure are respectively associated with the larger residue sizes of Y80, F84, F87, and F91 in mPGES-1^(MGST1) (Figure 5).

The residue-by-residue comparison shows that according to the model reported by Xing *et al.*, the Y130I mutation should not significantly affect the structure and catalytic activity of the trimer since the Y130 side chain is oriented toward the phospholipid membrane. This result is in contrast with our previous experiment data (and our computational study) showing that the Y130I mutation significantly decreases the catalytic activity of the enzyme by disrupting the stability of the trimeric enzyme.^{23, 35} The crystal structure of mPGES-1 shows that the Y130 has a hydrophobic interaction with L39 of the second mPGES-1 subunit, which is consistent with our mPGES-1 trimer model. Our CAS study and the biochemical data obtained for the N74A mutant reveal the importance of the residue and the consequence of the mutation of this residue to alanine. The mPGES-1 crystal structure also shows the interaction of N74 side chain with GSH. In contrast, according to the model reported by Xing *et al.*, N74 residue does not interact with GSH. Summarized in Table 5 are the crucial features observed in our mPGES-1 trimer model, in comparison with those in the mPGES-1 trimer model built by Xing *et al.* and the crystal structure of mPGES-1. The comparison reveals that our mPGES-1 model built based on the crystal structures of both MGST1 and ba3-cytochrome c oxidase is more reasonable than the mPGES-1 model constructed based on the crystal structure of the MGST1 trimer alone.

It should be pointed out that although our mPGES-1 model built based on the crystal structures of both MGST1 and ba3-cytochrome c oxidase is consistent with the crystal structure of mPGES-1 in the above-mentioned main features, there is a remarkable difference between our mPGES-1 model and the crystal structure concerning how GSH binds with mPGES-1. The difference is likely due to the low resolution (3.5 Å in plane) of the crystal structure, because a visual inspection of the binding structures clearly showed that the mPGES-1-GSH binding mode in our mPGES-1 model is more reasonable than that in the available crystal structure. According to the available low-resolution crystal structure of mPGES-1, there are some clearly unfavorable interactions between GSH and amino acid residues in the enzyme active site. First, the positively charged guanidinium side chains of R70 and R73 point toward each other and

are distant by only 2.62 Å (the difference between the positively charged nitrogen atoms). The orientations of R70 and R73 side chains are clearly unfavorable due to the charge repulsion and cannot allow the presence of hydrogen atoms on the guanidinium groups due to the short distance between the side chains. The conformational pose of GSH in the X-ray crystal structure was likely biased due to the unreasonable conformation of the residue side chains mentioned above. Further, the two carboxylate groups of GSH are distant by only 2.71 Å (the oxygen-oxygen distance) and oriented toward the carboxylate group of E77 with the distance as short as 3.07 Å (the oxygen-oxygen distance). These oxygen-oxygen distances in the crystal structure are too close to interact favorably and the repulsion between the negative charges should further destabilize the binding with GSH. Finally, as pointed out by the Jegerschold *et al.*,²⁵ the mPGES-1 crystal structure does not allow the docking of PGH₂. GSH and PGH₂ are expected to be between α -helices 1 and 4 that contribute to the formation of the active site pocket. For this reason, Jegerschold *et al.*²⁵ concluded that the mPGES-1 crystal structure represents a closed conformation of the enzyme. In comparison, our modeled 3D structure should represent an open conformation of mPGES-1.

Conclusion

Computational simulations based on our recently developed 3D model of mPGES-1 trimer structure, followed by site-directed mutagenesis and mPGES-1 activity assays, have led to a detailed understanding of mPGES-1 trimer interacting with substrate PGH₂ and cofactor GSH. Results obtained from the computational alanine scanning (CAS) reveal the contribution of each residue at the protein-ligand interaction interface to the binding affinity. The data from wet experimental tests are consistent with the computational predictions. The present work extends the previously identified important residues to include several crucial residues of mPGES-1 that enclose GSH and PGH₂. The per-residue decomposition of the GSH binding free energy reveals that the most important contributions arise from the polar and charged residues such as Asn74, Arg70, Arg122, and Arg126 while van der Waals interactions provide the driving force for binding with the highly conserved Tyr117 residue. In addition, the Asn74 and Arg126 residues also appear to influence the stabilization of the GSH and PGH₂ binding modes in the active site of mPGES-1. It has also been shown that for some mutations on mPGES-1, especially those associated with charged residues such as Arg67 and Glu77, a significant conformational rearrangement of the TM helices of the mPGES-1 trimer may take place, resulting in a drastic reduction in the catalytic activities of these mutants.

The detailed analysis of our mPGES-1 trimer model and other available 3D models (including an alternative homology model and a low-resolution crystal structure) suggests that our mPGES-1 trimer model (developed by using the crystal structures of both MGST1 and ba3-cytochrome c oxidase as templates) is more reasonable than the other homology model of mPGES-1 trimer constructed by simply using the low-resolution crystal structure of MGST1 trimer alone as a template. The available low-resolution crystal structure of mPGES-1 trimer represents a closed conformation of the enzyme and, thus, is not suitable for studying mPGES-1 binding with ligands. In comparison, our mPGES-1 trimer model represents a reasonable open conformation of the enzyme and is, therefore, promising for studying mPGES-1 binding with ligands in future structure-based drug design targeting mPGES-1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Views from two different orientations of the binding mode from the MD-simulated structures of GSH and PGH₂ in the catalytic active site of the mPGES-1 trimer.

Figure 2.

(Left) Determination of the Michaelis constant for PGH_2 . Data are plotted as a double reciprocal plot for the wild-type (WT) or mutated mPGES-1-GSH complex, with PGH_2 as the variable substrate. (Right) Experimental K_m (μM) values of substrate PGH_2 in the wild-type and mutated mPGES-1-GSH enzymes. The relative catalytic activity $(k_{cat}^{Mut}/k_{cat}^{WT})_{exp}$ represents the percentage of the mean values from wild-type mPGES-1.

Figure 3. Ribbons view of the mPGES-1 active site. (*Left*) Detailed view of the key residue Arg67 interacting with the Leu118 carbonyl backbone. (*Right*) Detailed view of the key residue Glu77 interacting with the Arg73' and Tyr80'' side chains of the second and third subunits of mPGES-1 trimer.

Figure 4.

(Top) Linear regression analysis between the $\Delta\Delta G_{\text{bind}}^{\text{GB}}$ values obtained by the computational alanine scanning and the experimental relative catalytic activity ($k_{\text{cat}}/k_{\text{cat}}$) values (in percentage). (Bottom) Scatter diagram of the experimental $\ln(k_{\text{cat}}/k_{\text{cat}})$ versus the $\Delta\Delta G_{\text{bind}}^{\text{GB}}$ relative binding free energies of GSH (kcal/mole).

Figure 5. Side view (A) and top view (B) of the human mPGES-1 trimer²³ core structure represented by the TM helices facing the core. The essential residues involved in the stability of the core are displayed in stick mode. Stick view (C) and ribbon diagram (D) of the structural core of human mPGES-1 trimer built by homology and based on the rat MGST-1 structure template.

Table 1

Components of the binding energy and relative binding free energy (kcal/mol) of the PGH₂ substrate to wild-type and mPGES-1^{mutant}-GSH complexes. Our experimental K_m (μ M) values for the wild-type and mutated enzymes are also reported. $\Delta\Delta G_{\text{exp}}$ (kcal/mol) = $-RT \ln(K_m^{\text{Mut}}/K_m^{\text{WT}})$, where $K_m^{\text{WT}}=215.5$ μ M, R is the ideal gas constant and T is the temperature in K.

PGH ₂	Wild-type	N74A	Y117A	R70A	R122A	R70A/R122A	E66A	R67A	H72A'	E77A	R70A/Y117A	Y117S	T129V
$\Delta\Delta E_{\text{ELE}}$	-381.50	2.28	-0.37	18.66	18.44	37.11	-14.87	14.85	9.27	-36.55	18.71	1.30	55.15
$\Delta\Delta E_{\text{VDW}}$	-48.94	1.35	0.35	0.10	0.25	0.35	-0.36	-0.36	2.87	0.13	0.07	0.35	-4.59
$\Delta\Delta G_{\text{GAS}}$	-430.44	3.63	-0.01	18.76	18.70	37.46	-15.23	14.48	12.15	-36.42	18.78	1.65	50.56
$\Delta\Delta G^{\text{PB}}_{\text{SUR}}$	-4.5	-0.05	0.00	0.01	0.02	0.02	0.01	0.00	-0.16	0.01	0.06	0.10	0.09
$\Delta\Delta G^{\text{PB}}_{\text{CAL}}$	405.11	2.07	0.37	-17.97	-17.94	-35.95	15.28	-14.50	-6.97	37.20	-18.41	-0.66	-49.68
$\Delta\Delta G^{\text{PB}}_{\text{SOL}}$	400.61	2.02	0.37	-17.96	-17.92	-35.93	15.28	-14.49	-7.13	37.21	-18.35	-0.56	-49.59
$\Delta\Delta G^{\text{PB}}_{\text{ELE}}$	23.61	4.35	0.01	0.70	0.50	1.16	0.40	0.35	2.30	0.65	0.30	0.64	5.47
$\Delta\Delta G^{\text{PB}}_{\text{bind}}$	-29.82	5.64	0.35	0.79	0.77	1.52	0.04	-0.02	5.01	0.78	0.42	1.08	0.96
$\Delta\Delta G^{\text{GB}}_{\text{SUR}}$	-6.47	-0.08	-0.01	0.00	0.02	0.02	0.00	0.00	-0.24	0.00	0.07	0.13	0.12
$\Delta\Delta G^{\text{GB}}$	374.57	1.18	0.64	-18.37	-18.38	-36.77	15.54	-14.48	-7.14	36.88	-18.32	-0.86	-49.83
$\Delta\Delta G^{\text{GB}}_{\text{SOL}}$	368.10	1.10	0.63	-18.37	-18.36	-36.75	15.54	-14.49	-7.38	36.88	-18.25	-0.73	-49.71
$\Delta\Delta G^{\text{GB}}_{\text{ELE}}$	-6.93	3.46	0.27	0.29	0.06	0.34	0.67	0.36	2.13	0.33	0.39	0.44	5.32
$\Delta\Delta G^{\text{GB}}_{\text{bind}}$	-62.34	4.73	0.62	0.39	0.33	0.71	0.31	0.00	4.77	0.46	0.53	0.92	0.85
$K_m(\text{our Exp.})$		464.30	534.50	296.00	372.00	473.70	-	-	-	-	-	283.20	384.20
$\Delta\Delta G_{\text{exp}}$		0.46	0.54	0.19	0.32	0.47	-	-	-	-	-	0.16	0.34

Table 2

Components of the binding energy and relative binding free energy (kcal/mol) of the GSH cofactor to wild-type and mPGES-1^{mutant} complexes. Our experimental values of the relative catalytic activity ($k_{cat}^{mut}/k_{cat}^{WT}$)_{exp} represent the percentage of the mean values from wild-type mPGES-1.

GSH	Wild-type	N74A	Y117A	R70A	R122A	R70A/R122A	E66A	R67A	H72A'	E77A	R70A/Y117A	Y117S	T129V
$\Delta\Delta E_{ELE}$	-452.81	17.51	0.92	54.01	24.01	79.81	-33.85	23.39	6.71	-56.87	53.34	17.59	1.19
$\Delta\Delta E_{VDW}$	-24.59	-0.67	1.80	3.17	1.84	5.00	2.37	-0.50	0.47	0.30	4.53	1.34	-0.54
$\Delta\Delta G_{GAS}$	-477.4	16.84	2.73	57.18	25.85	84.81	-31.48	22.89	7.18	-56.57	57.88	18.93	0.65
$\Delta\Delta G^{PB}_{SUR}$	-3.18	-0.01	-0.06	0.31	0.17	0.48	-0.02	-0.03	0.05	-0.04	0.22	-0.24	-0.01
$\Delta\Delta G^{PB}_{CAL}$	468.12	-12.14	0.26	-56.57	-25.72	-82.70	33.24	-22.11	-5.59	57.77	-49.58	-12.31	0.11
$\Delta\Delta G^{PB}_{SOL}$	464.94	-12.15	0.20	-56.26	-25.55	-82.22	33.23	-22.14	-5.54	57.73	-49.36	-12.55	0.10
$\Delta\Delta G^{PB}_{ELE}$	15.30	5.38	1.19	-2.55	-1.70	-2.88	-0.59	1.29	1.13	0.91	3.77	5.29	1.31
$\Delta\Delta G^{PB}_{bind}$	-12.46	4.69	2.93	0.92	0.30	2.59	1.75	0.75	1.64	1.16	8.52	6.38	0.75
$\Delta\Delta G^{GB}_{SUR}$	-4.18	-0.01	-0.09	0.45	0.25	0.70	-0.02	-0.04	0.08	-0.06	0.32	-0.35	-0.01
$\Delta\Delta G^{GB}$	462.13	-12.60	-0.17	-56.83	-25.78	-83.03	32.16	-22.70	-5.82	57.30	-51.49	-13.47	-0.23
$\Delta\Delta G^{GB}_{SOL}$	457.55	-12.61	-0.26	-56.38	-25.54	-82.33	32.14	-22.75	-5.74	57.24	-51.17	-13.82	-0.24
$\Delta\Delta G^{GB}_{ELE}$	9.31	4.92	0.77	-2.81	-1.77	-3.21	-1.68	0.70	0.90	0.44	1.86	4.13	0.97
$\Delta\Delta G^{GB}_{bind}$	-19.85	4.23	2.47	0.80	0.31	2.48	0.66	0.14	1.44	0.67	6.71	5.11	0.41
$(K_{cat}^{mut}/K_{cat}^{WT})_{exp}$ %	-	16(±)	24(±)	60(±)	78(±)	24(±)	53(±)	0(±)	32(±)	0(±)	7(±)	14(±)	88(±)

(±) Our Exp.;

(%) see reference (26)

Table 3

Binding energy decomposition (kcal/mol) to PGH₂ and mPGES-1-GSH from the mPGES-1-GSH-PGH₂ complex and to GSH and mPGES-1 from the mPGES-1-GSH complex.

	ΔE_{vdW}	ΔE_{elec}	ΔG_{gas}	ΔG_{sol}^{GB}	ΔG_{bind}
PGH ₂	-24.5	-190.6	-215.1	188.8	-26.4
mPGES-1-GSH	-24.3	-190.7	-215.3	179.3	-36.0
mPGES-1-GSH-PGH ₂	-48.8	-381.3	-430.4	368.8	-62.4
GSH	-12.6	-226.0	-238.6	237.0	-1.6
mPGES-1	-12.4	-226.1	-238.5	220.5	-18.0
mPGES-1-GSH	-24.7	-452.1	-477.0	457.6	-19.6

Table 4

Components of the decomposition of ΔG_{bind} (kcal/mol) on a per-residue basis for the interaction of GSH with mPGES-1 (*left*) and the interaction of PGH₂ with mPGES-1-GSH (*right*). The key residues are displayed in bold.

Interaction of PCH ₂ with mPGES-1-GSH											
Interaction of GSH with mPGES-1											
	ΔE_{vdW}	ΔE_{ele}	ΔG_{gas}	$\Delta G_{\text{GB}_{\text{sol}}}$	ΔG_{bind}		ΔE_{vdW}	ΔE_{ele}	ΔG_{gas}	$\Delta G_{\text{GB}_{\text{sol}}}$	ΔG_{bind}
Glu66	-0.2	16.2	16.0	-15.8	0.3	Glu66	0.0	7.8	7.8	-7.7	0.1
Arg67	-0.1	-11.3	-11.4	11.3	-0.1	Arg67	0.0	-7.1	-7.1	7.1	-0.1
Arg70	-1.3	-26.7	-28.0	25.9	-2.2	Arg70	-0.1	-8.8	-8.9	9.0	0.1
Arg73	-1.6	-37.3	-39.0	33.9	-5.1	Arg73	-0.3	-15.1	-15.4	14.6	-0.8
Asn74	-0.3	-8.6	-8.8	4.8	-4.0	Asn74	-1.0	-0.3	-1.3	0.1	-1.3
Glu77	-1.0	27.0	26.0	-26.4	-0.4	Glu77	-1.0	20.5	19.5	-19.5	0.0
Tyr117	-1.0	-0.5	-1.5	0.7	-0.8	Tyr117	-0.2	-0.4	-0.6	0.4	-0.2
Leu121	-17.7	-0.3	-2.0	1.1	-0.9	Leu121	-0.2	0.1	-0.1	0.0	-0.1
Arg122	-1.7	-11.6	-13.2	12.4	-0.8	Arg122	-0.7	-8.1	-8.8	8.8	0.0
Arg73'	-0.4	-28.8	-29.3	27.4	-1.9	Arg73'	-1.3	-17.5	-18.8	16.6	-2.2
p^{GSH} sub-pocket						p^{GSH} sub-pocket					
Thr78	-0.1	-0.3	-0.4	0.5	0.1	Thr78	-1.2	0.3	-0.9	-0.8	-1.7
Arg110	0.0	-13.4	-13.4	13.4	0.0	Arg110	1.5	-49.0	-47.6	35.7	-11.9
Ile125	-0.2	-0.3	-0.5	0.3	-0.2	Ile125	-1.7	0.8	-0.9	-1.1	-2.1
Arg126	-0.1	-11.9	-12.0	11.9	-0.1	Arg126	-1.4	-14.2	-15.6	13.5	-2.1
Thr129	0.0	-0.3	-0.3	0.3	0.0	Thr129	0.2	-6.4	-6.2	3.9	-2.4
Lys26'	0.0	-19.0	-19.0	18.8	-0.3	Lys26'	-0.6	-25.8	-26.4	24.7	-1.7
Leu69'	-0.4	1.1	0.7	-1.3	-0.6	Leu69'	-2.1	1.4	-0.7	-0.5	-1.2
His72'	-0.3	-2.4	-2.7	2.3	-0.4	His72'	-3.4	-3.7	7.1	2.7	-4.4
p^{GSH_2} sub-pocket						p^{GSH_2} sub-pocket					

Table 5

Residue by residue comparison of mPGES-1 structure between our model, Xing *et al.*²⁴ model, and crystal structure (pdb: 3dww.pdb).

mPGES-1 Crystal structure	Our mPGES-1 Model	Xing's mPGES-1 model
R38: salt bridge with D64 and GSH	R38: salt bridge with D64	R38: salt bridge with GSH
R40: Oriented toward the membrane	R40: Oriented toward the membrane	R40: salt bridge with carboxylate of GSH
E66: salt bridge with R70	E66: salt bridge with R70	E66: salt bridge with R70 and GSH
R70: H-bond with GSH	R70: H-bond with GSH	R70: salt bridge with E66
H72: close to GSH	H72: Dip.-quad. Interaction with PGH2	H72: H-bond with GSH
R73: salt bridge with GSH	R73: salt bridge with GSH	Unclear
N74: H-bond with GSH	N74: H-bond with GSH	No interaction
E77: close to GSH	E77: salt bridges with R73 and R73'	Unclear
R110: H-bond with GSH (PGH2 is not present in the crystal)	R110: salt bridge with PGH2	R110: buried and does not have contact with GSH and/or PGH2
R126: oriented toward GSH	R126: oriented toward PGH2	R126: salt bridge with GSH
K26-E77: distance 10.9Å (no interaction)	K26-E77: distance 8.7Å (no interaction)	K26-E77: Salt bridge (2.9Å)
F82/F103/F106 : strong hydrophobic contacts, perfect stacking	F82/F103/F106 : strong hydrophobic contacts, perfect stacking	F82/F103/F106 : strong hydrophobic contacts, perfect stacking