Glutathione Transferase Classes Alpha, Pi, and Mu: GSH Activation Mechanism

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Received: June 11, 2010; Revised Manuscript Received: August 24, 2010

Since the early 1960s, glutathione transferases (GSTs) have been described as detoxification enzymes. In fact, GSTs are the most important enzymes involved in the metabolism of electrophilic xenobiotic/endobiotic compounds. These enzymes are able to catalyze the nucleophilic addition of glutathione (GSH) sulfur thiolate to a wide range of electrophilic substrates, building up a less toxic and more soluble compound. Cytosolic classes alpha, pi, and mu are the most extensively studied GSTs. However, many of the catalytic events are still poorly understood. In the present work, we have resorted to density functional theory (DFT) and to potential of mean force (PMF) calculations to determine the GSH activation mechanism of GSTP1-1 and GSTM1-1 isoenzymes. For the GSTP1-1 enzyme, we have demonstrated that a water molecule, after an initial conformational rearrangement of GSH, can assist a proton transfer between the GSH cysteine thiol (GSH-SH) and the GSH glutamate alpha carboxylate (GSH-COO^-) groups. The energy barrier associated with the proton transfer is 11.36 kcal·mol^{-1}. The GSTM1-1 enzyme shows a completely different behavior from the previous isoenzyme. In this case, two water molecules, positioned between the GSH-SH and the ξ N atom of His107, working like a bridge, are able to promote the proton transfer between these two active groups with an energy barrier of 7.98 kcal·mol^{-1}. All our results are consistent with all the enzymes kinetics and mutagenesis experimental studies.

Introduction

The cell metabolism of electrophilic compounds highly depends on glutathione transferases (GSTs).[1] We focus our study on the GSTs' cytosolic classes alpha, pi, and mu. These enzymes can be homodimers or heterodimers (if both subunits are present in the same class of isozymes[2]). Each subunit has two active centers, a G-site and an H-site, and are composed of a distinct N-terminal alpha/beta domain, which adopts the thioredoxin fold, followed by a C-terminal all-helical domain.[3] The G-site is only completed after dimerization, because it is located in a cleft between the N-terminal domain of one subunit and the C-terminal domain of the other. It is mainly built by conserved residues and shows a high specificity for the substrate GSH. The H-site is a nonconserved pocket, allowing the binding of a vast spectrum of electrophilic toxic compounds, and is found in the C-terminal domain. GSTs are catalytically able to perform a nucleophilic addition of the sulfur thiolate of GSH to a wide range of electrophilic compounds, producing a conjugate which is less toxic and more soluble and can be easily eliminated from the organism. The product (GSR) release, controlled by the C-terminal domain, is the catalytic rate limiting step for fast substrates.[4,5]

After binding to the conserved G-site pocket, the GSH cysteine thiol group (GSH-SH) pK_a drops from 9.1 to ~6.2−6.6[6,21] and is subsequently deprotonated. Even though GSTs are being studied since the early 1960s, this GSH activation is still a matter of debate. For GSTA1-1, it was shown that the GSH glutamyl alpha carboxylate (GSH-COO^-) group and the G-site water molecules are important for activation.[6−13] Recently, we proposed a GSH activation mechanism for this class consistent with the known data.[14] Our studies showed that a water molecule promotes a proton transfer from the GSH-SH to the GSH-COO^-, with an activation energy of 13.39 kcal·mol^{-1}, after prior conformational rearrangement of GSH ($\Delta G = -1.62$ kcal·mol^{-1}). Studying the same isoenzyme, we were also able to further highlight the importance of water molecules as promoters of GSH activation.[15] We confirmed the existence of water coordination spheres around the GSH-SH and the GSH-COO^- groups and demonstrated that, in the case of the enzyme GSTA1-1, for residue-68 mutants, known for having a low catalytic efficiency, impressive changes in this water arrangement are observed.

What about the GSTM1-1 and GSTP1-1 GSH activation mechanism? The study of these isoenzymes reveals itself of great importance. Even though GSTM1-1 is a major liver GST,[16] 50% of the population are *hGSTM1-null* and therefore do not express this enzyme.[17,18] Extensively investigated, this *hGSTM1-nul* genotype has been highly associated with innumerous types of cancer and human diseases.[19−57] On the other hand, GSTP1-1 is known for being overexpressed in liver cancer cells[58,59]and has been implicated in the development of drug resistance in tumoral cells.[60−66] Additionally, the common GSTP1-1 Ile105Val mutant has been associated with the development of different types of cancer and human diseases.[31,67−78] In Figure 1 we can observe the crystallographic different G-sites of GSTA1-1, GSTP1-1, and GSTM1-1. In all three enzymes, a Tyr residue is hydrogen bonded with the GSH-SH; a charged residue, Arg or Lys, is hydrogen bonded with the GSH glycyl carboxylate; a Gln residue is stabilizing the GSH glutamyl amino group; a hydroxyl side chain group of a Ser or Thr is hydrogen bonded with the GSH-COO^- group. Focusing on the isoenzymes GSTA1-1 and GSTP1-1, we can conclude that the major difference between their G-site is the position of Arg and Glu residues.

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10.1021/jp1053875
Published on Web 09/20/2010

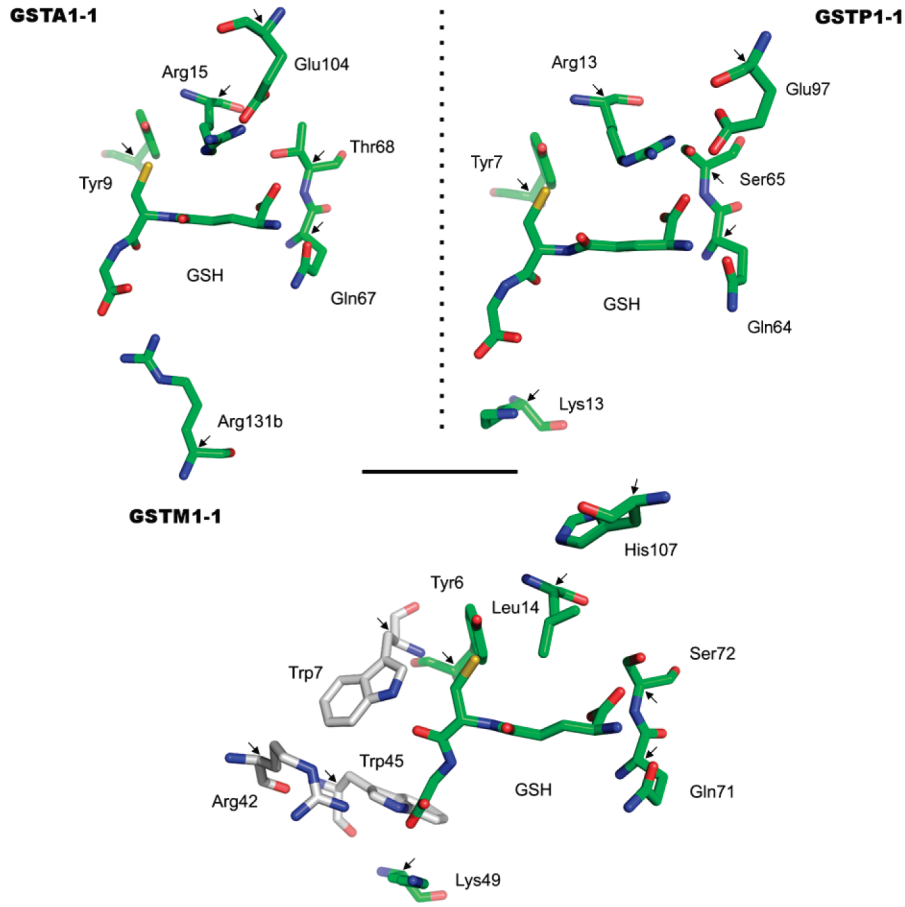

Figure 1. Relevant G-site active center amino acids of GSTM1-1 (PDB-1XW6), GSTP1-1 (PDB-6GSS), and GSTA1-1 (PDB-1PKW). The residues with carbons colored in green were later used as a model of the G-site in the study of the water proton transfer between the GSH-SH and the GSH-COO^-. The residues with carbons colored in gray were added to the GSTM1-1 G-site model in the study of the water proton transfer between the GSH-SH and the ξ N atom of His107. Arrows point the atoms fixed during the linear scans.

In GSTA1-1, Arg15 (a highly conserved residue in alpha GSTs) is involved in a salt bridge with Glu104. In the R15A and R15H mutants the specific activity is 200- and 400-fold lower, respectively, when compared with the wild-type enzyme.[79] In a recent study, to elucidate the catalytic role of Arg15, we analyzed the activation energy for the thiol deprotonation and the structural details associated with the mutants R15A, R15Rε,η-c (an Arg residue with the ε,η-nitrogens substituted by carbons), and R15Rneutral (a neutral Arg residue due to the addition of a hydride in the ζ-carbon).[80]

We concluded that the charged residue Arg15, positioned in the middle of GSH, dictates the arrangement of GSH in the active center by establishing a strong ion−dipole interaction and a hydrogen bond with the GSH cysteine main chain. For R15Rneutral mutant, hydrogen bond interactions are still possible to be established between the residue 15 side-chain ε,η-nitrogens atoms and the GSH cysteine main-chain carbonyl group. But, like occurred with the other mutants, eliminating the positive charge changed the orientation of residue 15 leading to a new, not catalytically efficient, GSH arrangement. The experimental data available for the mutant R15A supports the different GSH arrangement observed for the mutants, as K_MGSH is 10-fold increased relatively to wild-type enzyme.[79] In GSTP1-1 we still have an Arg (Arg13) residue involved in a salt bridge with a Glu (Glu97), however these residues are positioned differently in the G-site. Nevertheless, experimental studies proved that when GSTP1-1 Arg13 is mutated to a neutral residue, activity drops to values of a nonenzymatic reaction,[81] such as when Arg15 is mutated in GSTA1-1.[79] If we compare the G-site of GSTM1-1 with the other isoenzymes, significant differences can be found. GSTM1-1 Trp7 and Trp45 establish strong hydrogen bonds with GSH, not seen in GSTA1-1. The role of Arg42 in GSTM1-1 is not so evident. We will further discuss this, later in the paper. GSTM1-1 has Leu14 in the position of GSTA1-1 Arg15 residue and the residue His107 over it. Experimental studies demonstrated that, when GSTM1-1 His107 is mutated to a serine, to mimic the homologous less efficient GSTM4-4, the catalytic activity drops and the GSH thiol pK_a rises from 6.3 to 7.8.[82] When GSTM4-4 Ser107 is mutated to His, the catalytic activity rises and becomes identical to the one observed for wild-type GSTM1-1. The K_m values do not suffer significant changes. The same studies also showed that when Tyr6 is mutated to a phenylalanine the catalytic activity drops but, contrarily to what happens for GSTA1-1[79] and GSTP1-1,[83] the pK_a of the GSH-SH is unchanged. It was proposed that His107 could work as a base in the GSH activation.

In the present work, after an exhaustive computational study and in agreement with all the experimental data available, we propose the GSTP1-1 and GSTM1-1 GSH activation mechanisms. The G-site pockets of the three isoenzymes have significant similarities. Initially, we studied the hypothesis of GSTP1-1 and GSTM1 having similar GSH activation mechanisms to that already established for GSTA1-1.[14] However, we noticed that GSTM1-1 also shows some structural differences that are consistent with the residue His107 acting as a base and promoting the GSH activation. Therefore, for this isoenzyme, we also investigated an alternative mechanism in which two

water molecules, positioned between the GSH-SH and the ξ N atom of His107 imidazole ring, work as a bridge in assisting the transfer of the proton between these two groups.

Methodology

Overall Strategy. An initial study has allowed us to find out that the three isoenzymes, GSTA1-1, GSTP1-1, and GSTM1-1, do not share a similar GSH activation mechanism, with a water molecule assisting a proton transfer between the GSH cysteine thiol (GSH-SH) and the GSH glutamate alpha-carboxylate (GSH-COO^-) group, after the occurrence of an initial conformational rearrangement of GSH, just as it happens with GSTA1-1.[14] To perform this initial study, we first had to calculate the free energy associated to the GSH conformational rearrangement, from a more extended conformation to a more closed conformation. Accordingly, we have calculated the corresponding potentials of mean force (PMF), for both enzymes GSTP1-1 and GSTM1-1, using the umbrella sampling method. Subsequently, starting from the final PMF structure of the isoenzymes, G-site active center models were built for both enzymes. We then performed linear scans, on those models, of the water proton approach to the most suitable GSH-COO^- group oxygen atom. From the scan, structures of the stationary points were obtained and the respective proton transfer activation energies for both enzymes were calculated.

As, in the GSTM1-1 case, this study did not produce reliable numbers for the resulting mechanistic activation energies, we had to follow another strategy able to result in the establishment of the correct GSH activation mechanism. As explained above, some studies suggest that His107 can work as a base, receiving the GSH-SH proton. To test this hypothesis, we begin by conducting a long molecular dynamics (MD) study of the GSTM1-1 complexed with GSH in aqueous solvent. With a MD reference structure, we built a model of the G-site active center in which we kept two waters between the active groups. Finally, a linear scan of the active center water proton approach to the His107 imidazole ξ N atom was performed. From it, structures of the stationary points were obtained and the proton transfers activation energy was calculated.

The full description of the overall strategy follows.

Conformational Rearrangement of GSH. The crystallographic structures of the enzymes complexed with GSH were obtained from the Protein Data Bank (PDB)[84] (GSTM1-1-1XW6;[85] GSTP1-1-6GSS[86]). A water molecule was later added and placed between the GSH-SH and the GSH-COO^- groups in both enzymes. All the MD conditions and parameters are identical to the ones used in the previous study of GSTA1-1 activation mechanism.

Potential of Mean Force Calculations. The PMF represents the Gibbs energy change, ΔG, as a function of a coordinate of the system. In our study, the distance δ between the hydrogen (atom A) and oxygen (atom B) atoms was taken as the reaction coordinate (Figure 2A). It should be noted also that the position of the water molecule was constrained. The PMF calculation was performed with the umbrella sampling method.[96] In the umbrella sampling method a series of simulation windows are performed along a reaction coordinate and each window is restrained by imposing a harmonic umbrella biasing potential $U'(\delta)$,

$$U'(\delta) = \frac{1}{2}\kappa(\delta - \delta_0)^2$$

in which κ is the force constant.

All the parameters of the PMF calculation are identical to the ones used in the previous study of GSTA1-1 activation mechanism.

Proton-Transfer Study. On the basis of the final structure of the PMF calculation, we built two G-site models (GSTP1-1 141 atoms; GSTM1-1 210 atoms, Figure 1). The α-carbons of both isoenzymes G-site active centers were kept fixed during the proton transfer (Figure 1). The GSH glutamate α-amino group was included in the deprotonated state, because it is situated in the molecule|vacuum interface of the model, has no other residue nearby to compensate its charge, and is not directly involved with the reaction coordinate. To rationalize the computation time, we have resorted to the ONIOM hybrid method[98−100] in the geometry optimization. The high layer included GSH, the water molecule, and the atoms that directly interact with GSH and may play an important role in the proton transfer (GSTM1-1 77 atoms; GSTP1-1 78 atoms). Density functional theory (DFT) with the B3LYP functional[108,109] and the basis set 6-31G(d) was used in all geometry optimizations as implemented in Gaussian03.[110] The low layer included the rest of the model atoms that complete the G-site pocket. This layer was treated with the semiempirical method PM3MM.[111,112] Hydrogen atoms were used as link atoms at the truncated bonds. For each enzyme, a linear scan of the water proton approach to the GSH-COO^- group oxygen was performed. Approximate structures of the stationary points were taken from the scan and optimized, and their nature was confirmed by frequency calculations. The stationary points' final energies were later on recalculated using DFT with the functional B3LYP and the 6-311++G(2d,2p) basis set. To mimic the effect of the remaining protein environment, the C-PCM continuum model[113,114] with a dielectric constant of 4 was used. Zero-point corrections, and thermal and entropic effects, were also added to obtain the final free energies values (T = 310.15K, P = 1 bar).

GSTM1-1: Alternative Mechanism. This study can be divided in two stages. We started by conducting a long MD study (simulation of 50 ns with a time step of 2 fs) of the GSTM1-1 complexed with GSH. The MD conditions were identical to those used in the previous study of GSTA1-1 GSH activation mechanism, except for the production simulations time and the parameters directly related to the umbrella sampling method. The MD simulation, by following the time-dependent position of the backbone and side-chain atoms, is able to describe dynamical properties of the protein and so give important insights of it catalytic function. A reference structure resulted from this study, corresponding to the one more similar with the average MD simulation structure. In the second stage, starting with this MD simulation reference structure, we built a model of the G-site active center that included two water molecules positioned between the GSH-SH and the ξ N atom of the His107 imidazole ring (234 atoms, Figure 1). The GSH glutamate α-amino group was included in the deprotonated state and the α-carbons of the G-site active center were fixed during the proton transfer (Figure 1). To optimize the geometry of the model, we resorted to the ONIOM hybrid method.[98−100] The high layer included GSH, the atoms that directly interact with GSH, the water molecules and His107 (95 atoms B3LYP functional,[108,109] basis set 6-31G(d)), while the low layer includes the rest of the model (PM3MM[111,112]). Then a linear scan of the water proton approach to the His107 imidazole ξ N atom was performed (Figure 3, σ). From the scan, all structures for the stationary points were obtained. Later on, their final energies were recalculated with a higher theoretical level (B3LYP functional,[108,109] basis set 6-311++G(2d,2p)), a continuum

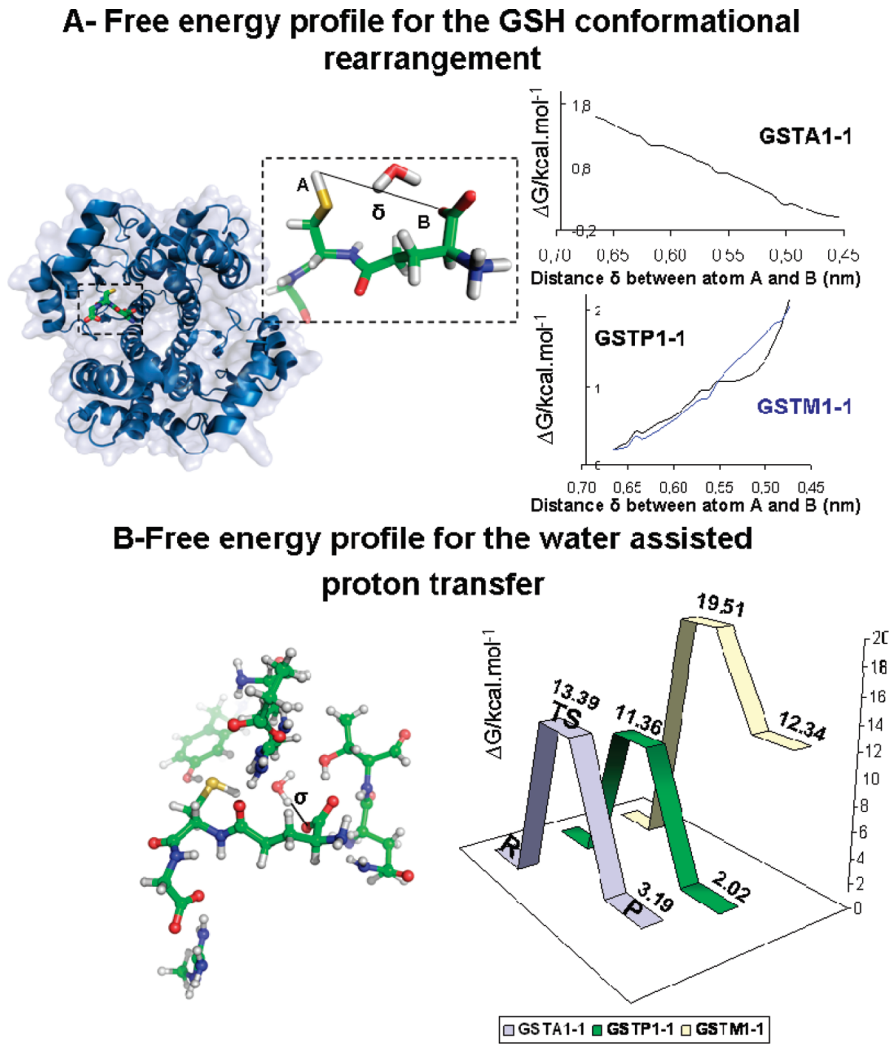

Figure 2. GSTA1-1, GSTP1-1, and GSTM1-1 water-assisted proton transfer mechanism. (A) PMF along the SH−COO$^-$ reaction coordinates. The graphs on the right-hand side represent the sum of the entire data obtained from the PMF forward and backward processes. On the left-hand side, a view of the GSTA1-1 complex with GST is used to highlight the reaction coordinate, δ. (B) Free energies of activation and reaction for the proton transfer between the GSH-SH and the GSH-COO$^-$ groups (functional B3LYP and basis set 6-311++G(2d,2p)). On the left-hand side, the G-site model of GSTA1-1 is shown to represent the distance σ, decreased at each scan point in each isoenzyme, as the reaction coordinate is explored.

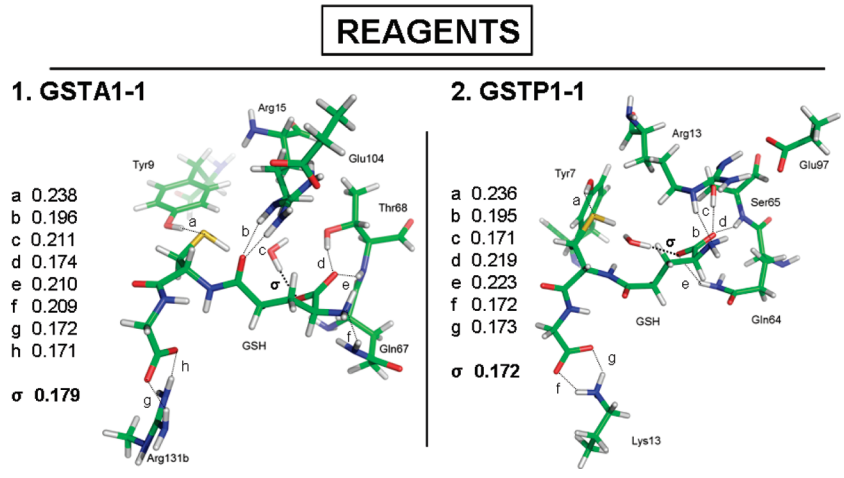

Figure 3. Reagents for the water-assisted proton transfer: (1) GSTA1-1 and (2) GSTP1-1. Relevant distances (nm) are shown.

model was used as an approximation to the effect of the remaining protein environment and zero-point corrections, and thermal and entropic effects were also obtained in order to obtain the final free energies values (T = 310.15K, P = 1 bar).

Results and Discussion

Figure 2 shows the results of the GSH structural rearrangement plus the proton transfer Gibbs energies for the three

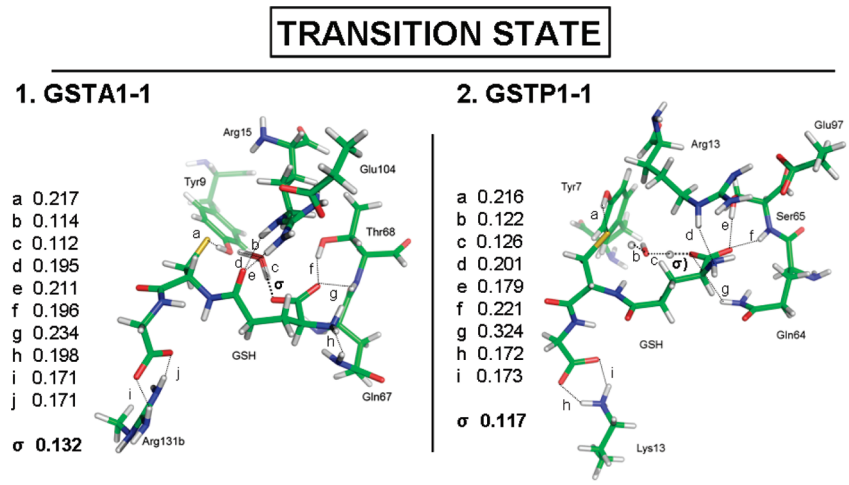

Figure 4. Transition state for the water-assisted proton transfer: (1) GSTA1-1 and (2) GSTP1-1. Relevant distances (nm) are shown.

isoenzymes. The GSTA1-1 data was taken from ref 14. The PMF curves (Figure 2A) show a minimal hysteresis, rejecting the possibility of systematic error and emphasizing the accuracy of the calculations. For GSTA1-1 the PMF calculation demonstrates that the bent GSH, with a water molecule bridging both active groups, is actually more stable than the normal opened GSH conformation ($\Delta G_{\text{rearrangement}} = -1.62$ kcal·mol^{-1}). The crystallographic structure we used shows a water molecule hydrogen bonded to the GSH-COO$^-$ group but not bridging the two GSH active groups. So GSH is in an open conformation. Possibly a small barrier may exist to move the water molecule to the bridging position. The PES surface for these rearrangements is usually very flat and with multiple minima, and surely not rate-limiting. On the other hand, for GSTP1-1 ($\Delta G_{\text{rearrangement}} = 1.92$ kcal·mol^{-1}) and for GSTM1-1 ($\Delta G_{\text{rearrangement}} = 1.68$ kcal·mol^{-1}) the normal open conformation is slightly more stable. Structural analysis showed that in GSTA1-1 the G-site Arg15 adopts a spatial arrangement that allows for intermolecular interactions with both the GSH-SH and the GSH-COO$^-$ groups, promoting the closed GSH conformation. In the last structure of the PMF calculation, the distance of Arg15 Nε to the GSH-SH sulfur atom and to the GSH-COO$^-$ oxygen atom is 0.393 and 0.398 nm, respectively.

Figure 2B shows the Gibbs activation and reaction energies associated with the proton-transfer studies. The barriers for GSTA1-1 and GSTP1-1, 13.39 and 11.36 kcal mol^{-1}, respectively, are values consistent with typical enzymatic reactions. The reactions for both isoenzymes are endergonic (GSTA1-1 $\Delta G_r = 3.19$ kcal mol^{-1}; GSTP1-1 $\Delta G^{\ddagger} = 2.02$ kcal mol^{-1}), resulting in strong nucleophiles. However, for GSTM1-1, the energy barrier associated with the proton transfer (19.51 kcal mol^{-1}) is too high for an enzymatic reaction. These results demonstrate that a positively charged residue in the G-site active center (Arg15 for GSTA1-1 and Arg13 for GSTP1-1) is essential in this GSH activation mechanism. At this point, for this isoenzyme, we focused only in the alternative GSH activation mechanism, which will be discussed subsequently in the text.

Figure 3 shows the structures of isoenzymes GSTA1-1 and GSTP1-1 stationary points.

In GSTA1-1, the Arg15 residue is bound to the GSH cysteine main chain by a strong hydrogen bond established between its Nε atom and the GSH carbonyl group (Figure 3.1, distance *b*), as well as through a strong ion−dipole interaction between its Nη atom and the same GSH carbonyl group (Figure 3.1, distance *c*). In GSP1-1, GSH is in a different arrangement. In this isoenzyme the Arg13 side-chain Nε atom is hydrogen bound to the GSH glutamyl amino group (Figure 3.2, distance *c*) and consequently the Gln64 side-chain amino group changed orientation toward the GSH-COO$^-$ group (Figure 3.2, distance *e*). If we compare the transition-state structures of isoenzymes GSTA1-1 and GSTP1-1, we can observe that only in the first a H3O$^+$ intermediary structure is formed (Figure 4.1, distances *b*,*c* and Figure 4.2, distances *b*,*c*). In GSTP1-1, the water proton has to be closer to the GSH-COO$^-$ oxygen atom (Figure 6.1, distance σ and Figure 6.2, distance σ) to promote the proton transfer. In the two enzymes a shortening of the hydrogen bond between Tyr9 and the nascent thiolate is observed (Figure 4.1, distance *a* and Figure 4.2, distance *a*).

Analyzing the products, we can make the following remarks (Figure 5). In GSTA1-1, we can observe that the Thr68 side chain, which was hydrogen bound to the GSH GSH-COO$^-$ oxygen atom, is now hydrogen bound to the water molecule instead (Figure 5.1, distance *d*). In GSTP1-1, the initial hydrogen bond established between the Ser65 side chain and the GSH GSH-COO$^-$ oxygen atom is maintained (Figure 5.2, distance *c*), most likely because of the different conformation of GSH. In the two isoenzymes, the amino acid Tyr9 is even closer to the sulfur atom, establishing a stronger ionic hydrogen bond interaction with it (Figure 5.1, distance *a* and Figure 5.2, distance *a*). The Tyr9 residue assumes the role of stabilizing the negative charge in the thiolate group.

In a recent study with GSTA1-1, we were able to further highlight the importance of water molecules as promoters of GSH activation.[15] We confirmed the existence of water coordination spheres around the GSH-SH and the GSH-COO$^-$ groups. In this present study, we performed an identical analysis for GSTP1-1. The results show that there are well-defined water coordination spheres around the GSH-SH and the GSH-COO$^-$ groups and that the pattern is identical to the one observed in GSTA1-1. The importance of water molecules for GSTP1-1 GSH activation is unequivocal. The corresponding radial distribution functions (RDF) can be seen in the Supporting Information.

GSTM1-1: Alternative Mechanism. As we have shown above, GSTM1-1 does not work through a GSH activation mechanism identical to the other two isoenzymes. Therefore, we analyzed the hypothesis of two water molecules, acting as a bridge, being able to assist the proton transfer from the GSH-SH group to the His107 ξ N atom. Figure 6 represents the structures and the free energies of the stationary points.

The activation energy, $\Delta G^{\ddagger} = 7.98$ kcal·mol^{-1}, is consistent with that of an enzymatic reaction. This is an endergonic

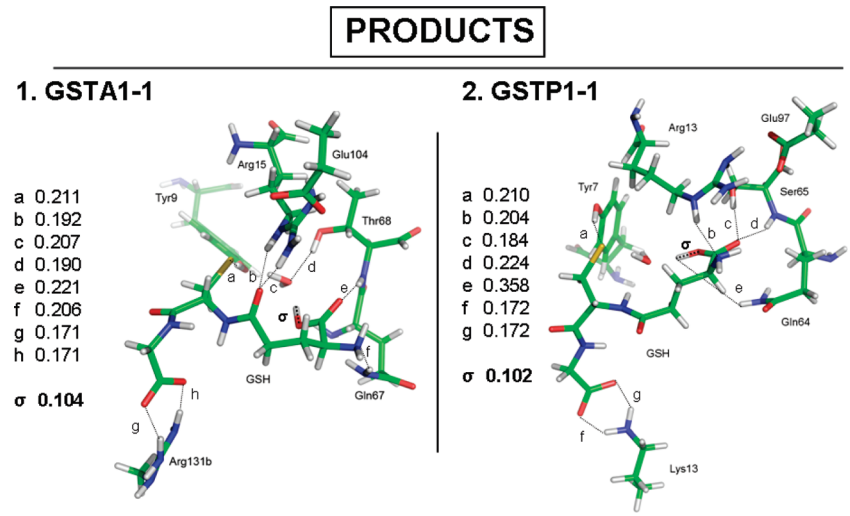

Figure 5. Products for the water-assisted proton transfer: (1) GSTA1-1 and (2) GSTP1-1. Relevant distances (nm) are shown.

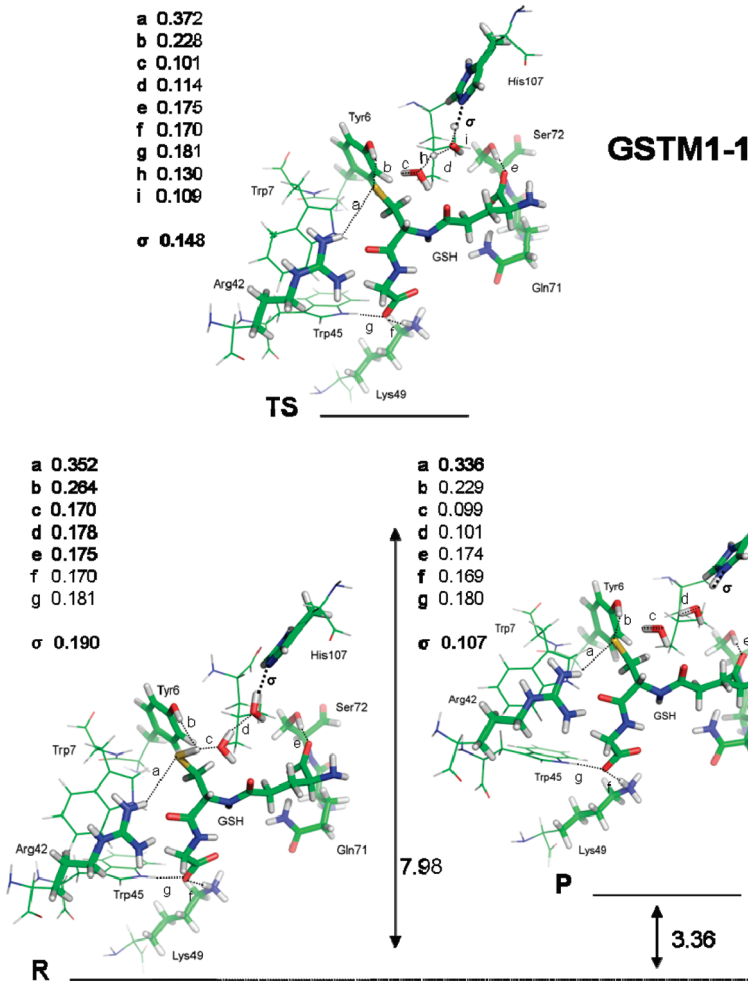

Figure 6. Free energy of the GSTM1-1 proton transfer (kcal·mol^{-1}). Relevant distances (nm) are shown.

reaction (ΔG_r = 3.36 kcal mol^{-1}) resulting in a strong nucleophile. The analyses of the MD simulation and the QM model system demonstrate that, unlike the crystallographic structure (Figure 1), the Arg42 side chain is orientated toward the GSH-SH group (Figure 6). To clarify the role of this residue in the catalysis, we built yet another G-site model without including Arg42 and the adjacent residues Trp7 and Trp49. Then, a scan for the approach of the water proton to the His107 ξ N atom was performed. The scan has revealed a barrier of 25.47 kcal·mol^{-1}, and the reaction proves to be very endergonic (11.09 kcal·mol^{-1}). Clearly the positive charge of Arg42 is fundamental to stabilize the negative charge that develops in the GSH-SH group. Accordingly, experimental studies indicate that the Tyr6 residue should not be the only one stabilizing the thiolate.[82] Contrarily to GSTA1-1[79] and GSTP1-1[83] isoenzymes, here when Tyr6 is mutated the catalytic activity drops but the pK_a of the GSH-SH is maintained.

To further support the water molecules' role in the GSTM1-1 GSH activation, RDFs were calculated for GSH-SH and the His107 imidazole ξ N atom, in relation to the water oxygen atoms (OW). Results showed that around the GSH-SH a well-defined water coordination sphere, composed of 4 water molecules, can be found. On the other hand, around the His107 imidazole ξ N atom, it is possible to observe two equally well-defined water coordination spheres. The first corresponds to exactly 2 water molecules and the second coordination sphere to 7 water molecules. The importance of the water molecules for GSTM1-1 GSH activation is unequivocal. The respective RDFs plots can be observed in the Supporting Information.

Conclusion

We have demonstrated that GSTA1-1 and GSTP1-1 share a similar GSH activation mechanism. Initially, in both enzymes, there must be a GSH conformational rearrangement so that the water molecule present in the G-site is able to interact directly with the GSH-SH and the GSH-COO^- groups. For GSTA1-1 this is an exergonic reaction (ΔG of -1.62 kcal$\cdot$mol^{-1}), because in the closed conformation the positive charged Arg15 is able to interact with both groups, GSH-SH and GSH-COO^-, stabilizing the structure. On the other hand, for GSTP1-1, the normal open conformation is more stable (ΔG of 1.92 kcal$\cdot$mol^{-1}). In the end, also for both enzymes, the water molecule acting as a bridge, is able to transfer the proton from the GSH-SH to the GSH-COO^- group. The energy barrier for this reaction is 13.39 kcal$\cdot$mol^{-1} for GSTA1-1 and 11.36 kcal$\cdot$mol^{-1} for GSTP1-1, which is in conformity with the experimental value obtained for the catalyzed GSH$-$CNDB conjugation, a common electrophilic substrate (GSTA1-1 $\Delta G^{\ddagger}$ 15.06 kcal$\cdot$mol^{-1} [7]; GSTP1-1 $\Delta G^{\ddagger}$ 15.38 kcal$\cdot$mol^{-1} [115]), and is consistent with the fact that the product release is rate-limiting. The GSTP1-1 Arg13 residue is positioned differently in this protein as opposed to GSTA1-1, and this seems to prevent a similar GSTA1-1 GSH arrangement.

GSTM1-1 cannot have a GSH activation mechanism identical to the other two isoenzymes, because the energy barrier for the proton transfer is too high for this type of enzymatic reactions ($\Delta G^{\ddagger} = 19.51$ kcal$\cdot$mol^{-1}). A positive charge residue in the G-site active center (Arg15 for GSTA1-1 and Arg13 for GSTP1-1) is essential in this GSH activation mechanism. For GSTM1-1 two water molecules, positioned between the GSH-SH and the ξ N atom of His107, working like a bridge, are able to promote the proton transfer between these two active center groups. The energy barrier of this reaction, $\Delta G^{\ddagger} = 7.98$ kcal$\cdot$mol^{-1}, is in agreement with the experimental value obtained for catalyzed GSH$-$CNDB conjugation, a common electrophilic substrate ($\Delta G^{\ddagger}$ 15.82 kcal$\cdot$mol-1 [82]), and is consistent with the fact that the product release is rate-limiting. This is an endergonic reaction ($\Delta G_r = 3.36$ kcal mol^{-1}) giving rise to a strong nucleophile. Arg42 is fundamental to stabilize the newly formed negative charge of the GSH-SH, because the removal of this conserved residue raises the energy barrier to values of a nonenzymatic reaction (25.47 kcal$\cdot$mol^{-1}).

Acknowledgment. We thank the FCT (Fundação para a Ciência e Tecnologia) for financial support linked to Project POCI/QUI/61563/2004 as well as for a Ph.D. grant SFRH/BD/32127/2006 for D.F.A.R.D.

Supporting Information Available: GSTP1-1 and GSTM1-1 radial distribution functions (RDFs) are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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JP1053875