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On the mechanism of microsomal prostaglandin E synthase type-2—A theoretical study of endoperoxide reaction with MeS^-

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ABSTRACT

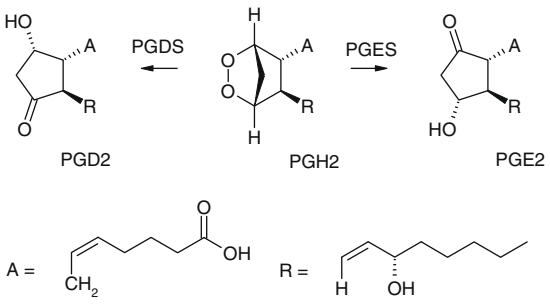
The reaction pathways of deprotonation versus nucleophilic substitution involving mPGES-2 enzyme catalysis were investigated by ab initio molecular orbital theory calculations for the reaction of methylthiolate with the endoperoxide core of PGH_2 and by the combined quantum mechanical molecular mechanical methods. The calculations showed that deprotonation mechanism is energetically more favorable than the nucleophilic substitution pathway.

Prostaglandin H_2 (PGH_2), produced from arachidonic acid by cyclooxygenases (COX-1 and COX-2) and peroxidase, is the primary precursor to all other biologically significant prostanoids.[1] Since PGH_2 readily undergoes non-enzymatic rearrangement to more stable prostaglandin D2 (PGD_2) and prostaglandin E2 (PGE_2) in aqueous media, or fragmentation to ketoaldehydes such as levuglandins D_2 and E_2,[2] biosynthesis of prostaglandins from PGH_2 is tightly controlled by enzymatic pathways. For instance, PGD synthases (PGDS) and PGE synthases (PGES) catalyze specifically isomerizations of PGH_2 to PGD_2 and PGE_2, respectively, as shown in Scheme 1.[3]

Both PGDS and PGES exist in several distinct forms. Hematopoietic PGDS found in peripheral tissues is a glutathione (GSH) requiring enzyme,[4] whereas lipocalin-type PGDS (lPGDS) located centrally is GSH-independent catalyzing biosynthesis of PGD_2 in the brain.[5] Similarly, there are at least three prostaglandin E synthases known to catalyze PGH_2 to PGE_2 isomerization. Both the cytosolic enzyme (cPGES)[6] and the membrane associated PGES (mPGES-1)[7] belong to glutathione *S*-transferase family, requiring GSH as co-factor. In addition, a GSH-non-specific membrane associated PGES has also been found and is named microsomal PGES type 2 (mPGES-2).[8]

A sulfhydryl group of cysteine, from either enzyme or GSH co-factor, has been proposed to play a key role in all known enzymes that catalyze isomerizations of PGH_2 to PGD_2 or PGE_2. This was manifested by loss of enzyme activity from critical Cys to Ala/Ser mutation of GSH-independent enzymes (mPGES-2[8b] and lPGDS[5d]) and GSH-dependency for the others. Furthermore, the crystal structures of hPGDS,[9] lPGDS,[10] mPGES-2,[11] and the 2D electron crystallography of mPGES-1[12] all revealed the presence of a sulfhydryl group near the catalytic site.

Since an ionized sulfhydryl (thiolate) can act either as a base or as a nucleophile, two different mechanistic pathways have been proposed for the enzymatic catalysis of the endo peroxide moiety of PGH_2 to a β-keto alcohol, as in PGD_2 and PGE_2.[5d,9a,11] One involves nucleophilic attack of the thiolate anion at one of the peroxide oxygen atom (S_N2 reaction), as shown in Scheme 2a, to form an



Scheme 1.

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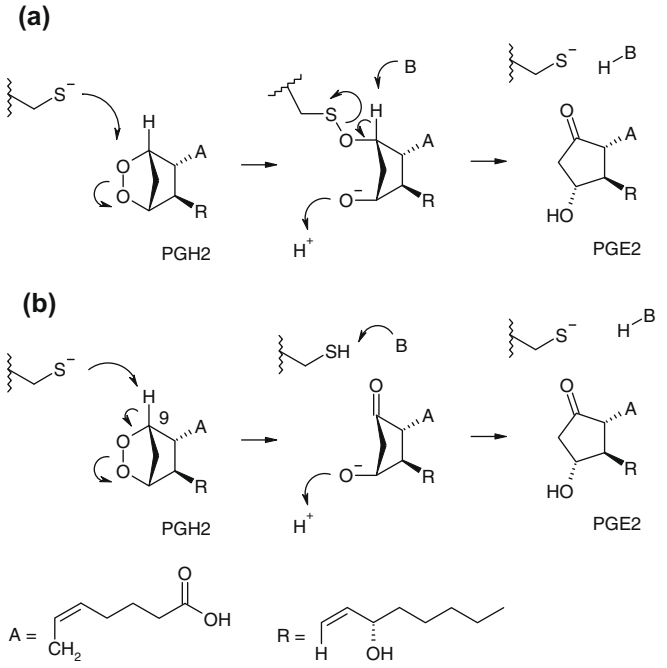

Scheme 2. Reagents: (a) mechanism of SN2 nucleophilic displacement of peroxide bond; (b) mechanism of deprotonation.

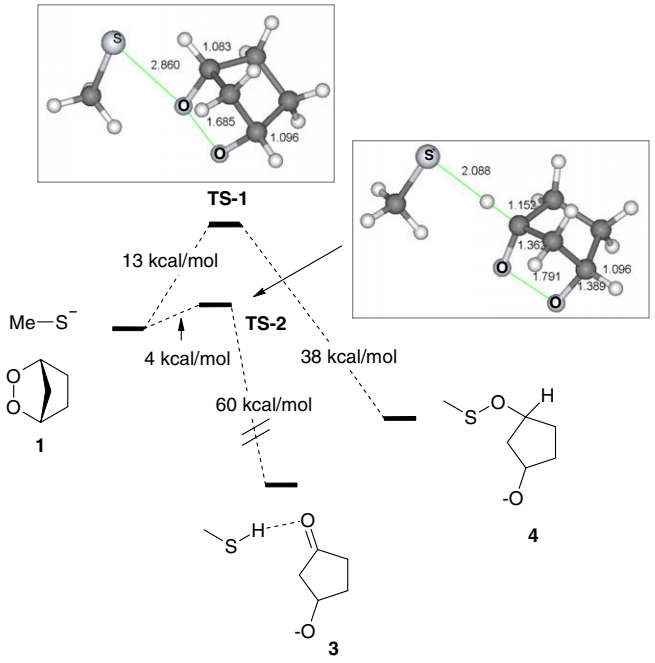

Figure 1. The transition structures and energetics for the deprotonation and nucleophilic substitution reaction of **1** with methylthiolate.

intermediate, which is then followed by deprotonation and S–O bond cleavage. This mechanism is analogous to the well known nucleophilic displacement of thiol-disulfide interchange reactions in biochemical transformations.[13] An alternative mechanism involved first deprotonation at H-C9 by the thiolate anion in concert with the cleavage of the peroxide bond to form β-keto alkoxide/alcohol as shown in Scheme 2b. Although there was evidence to support the deprotonation pathway,[11] the S_N2 nucleophilic displacement mechanism is often invoked in spite of limited knowledge about the energetics of these alternative pathways.

Chemically, the unstable endoperoxide moiety is susceptible to base-catalyzed fragmentation known as Kornblum–DeLaMare reactions.[14] Zagorski and Salomon examined in details of kinetic isotope effects for base-catalyzed fragmentation of deuterated PGH_2 endoperoxide core molecules. The observed primary kinetic isotope effects were consistent with the deprotonation mechanism.[15] For the cleavage of an O–O peroxide bond lacking a proton at the α-carbon, it may involve nucleophilic S_N2 attack on the dialkyl peroxides.[16] It is also known that dialkyl peroxides undergo cleavage by radical reactions or Lewis acid catalyzed reactions.[17] Similar radical reactions are likely involved in the degradation of endoperoxides catalyzed by heme containing enzymes such as PGI_2 synthase,[18] thromboxane synthase[19] or the GSH-heme bound mPGES-2 complex (mPGES-2 h) that converts PGH_2 to products other than PGE_2.[20]

To gain insights into the mechanism of mPGES-2 catalyzed transformation of PGH_2 to PGE_2, we carried out ab initio quantum mechanical (QM) calculations on the intrinsic potential energy profile for the reaction of methylthiolate with an endoperoxide of the PGH_2 core molecule. We also carried out a preliminary study on the energetics of mPGES-2 complexed with PGH_2 using a combined QM and molecular mechanical (MM) approaches. We report here computational studies on the deprotonation versus the S_N2 mechanism of PGH_2 to PGE_2 conversion catalyzed by mPGES-2.

For the reaction of methylthiolate with the endoperoxide core (**1** in Fig. 1), ab initio calculations were performed using the density functional theory at the B3LYP level[21] with the AUG-cc-pVTZ basis set[22] as implemented in the GAUSSIAN03 program.[23] Geometries of the reactants and intermediate/product were fully optimized. Transition structures (TS) were located and characterized to be first-order saddle points with single imaginary vibrational frequency. Figure 1 shows the optimized structures and the corresponding energetics.

The S_N2 nucleophilic displacement of an O–O bond by an O–S bond is thermodynamically favorable. The O–O bond in a dialkyl peroxide MeO–OMe is weak with calculated bond dissociation energy of only 39 kcal/mol, in comparison to that of 63 kcal/mol for an S–O bond in MeS–OMe.[24] Bach and co-workers also reported computational studies for the reaction (MeS^- + MeO–OMe→MeS–OMe + MeO^-) to be exothermic by 18 kcal/mol with a transition structure 3.6 kcal/mol above the isolated reactants.[25] By comparison, our calculations predict the S_N2 attack of MeS^- on O–O bond of endoperoxide **1** is exothermic by 25 kcal/mol with transition structure TS-1 that is 13 kcal/mol above the ion-molecule complex.

On the other hand, the deprotonation of **1** by a methylthiolate is predicted to be thermodynamically more favorable (by about 30 kcal/mol), with a reaction barrier of 9 kcal/mol lower than the aforementioned S_N2 reaction. The transition structure TS-2 is early as indicated by the slightly stretched H–C bond, but the O–O bond of 1.79 Å is elongated further than the O–O bond (1.69 Å) in the transition structure TS-1 for the S_N2 reaction. These calculations indicate that the deprotonation mechanism is energetically more favorable than the S_N2 mechanism for thiolate catalyzed endoperoxide rearrangement.

The mechanism of PGH_2 isomerization mediated enzymatically by PGES and PGDS can be significantly different from the enzyme free degradation of endoperoxide. The acceleration of reaction by the enzyme could be brought about by either direct contacts of the substrate and the active site through non-covalent interactions[26] or a change of reaction mechanism.[27] In a recent report on mPGES-1, a simple R126A or R126Q mutation of the enzyme abolished its PGES activity to exhibit a novel GSH-dependent reductase activity.[28] This surprising finding suggested that the S_N2 mechanism is more likely involved in its PGES activity, where the a common S–O intermediate can be followed by deprotonation and S–O bond cleavage to PGE_2 from the native enzyme or reduced by GSH to $PGF_2\alpha$. from the R126A/Q mutants.[28]

To simulate and probe the effect of the enzymatic environment of mPGES2, we performed a preliminary study of deprotonation

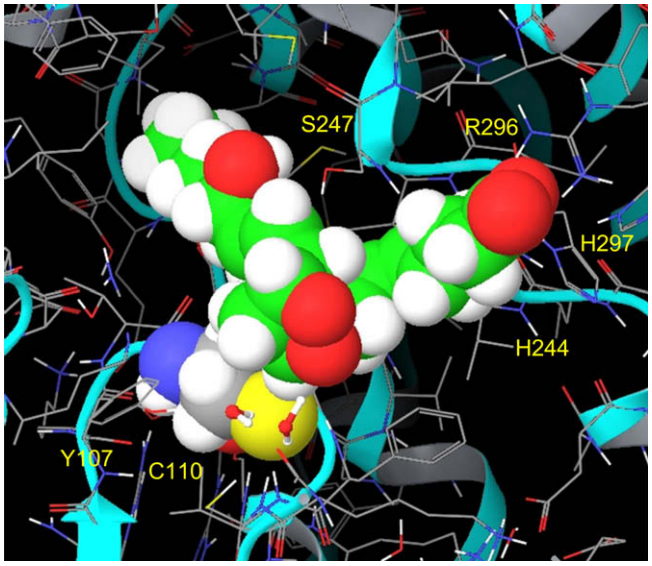

Figure 2. The QM/MM optimized structure of mPGES-2 and PGH2 complex. PGH2 and Cys110 are shown in space-filling model, water molecules are shown as ball-and-stick.

versus S_N2 reactions using QM/MM approach as implemented in the Qsite module of software from Schordinger.[29] Similar to one of the two binding modes proposed by Takusagawa and co-workers,[11] PGH_2 was docked into the crystal structure of mPGES-2 (PDB code: 1z9h). Due to the size of system, only PGH_2 and residues Thr109-Cys110-Pro111 and Ser247 were treated by QM using DFT (B3LYP) and the LACVP* basis set.[30] Cys110 was selected since its thiolate is involved in the catalytic reaction. The neighboring Thr109 and Pro111 are included as required by the Qsite program. Ser247 also constituted to the QM region for its proposed H-bond interactions with 15-OH in ω-chain of PGH_2.[11] Other residues (Cys113 and Phe112) that interact with Cys110 were treated by molecular mechanics with the OPLS force field.[31] Crystallographic waters, including those forming a hydrogen bond network with Tyr107, were maintained and treated by molecular mechanics. The structure of mPGES-2 complexed with the docked PGH_2 substrate was fully optimized as shown in Figure 2 using the QM/MM method. For the thiolate anion to approach the peroxide oxygen in S_N2 reaction, the PGH_2 substrate has to be brought deeper into the hydrophobic binding site. On the other hand, the thiolate anion of Cys110 is predisposed in the enzyme-substrate complex to abstract the H-C9 proton for the deportation pathway.

In summary, ab initio calculations on thiolate catalyzed endoperoxide fragmentation showed the deprotonation pathway is intrinsically more favorable than the nucleophilic S_N2 displacement of peroxide bond. Our primitive QM/MM calculations of mPGES-2 complex with PGH_2 substrate also suggested deportation mechanism is more favorable than the nucleophilic attack mechanism, but a definitive answer requires further detailed experimental or computational studies.

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