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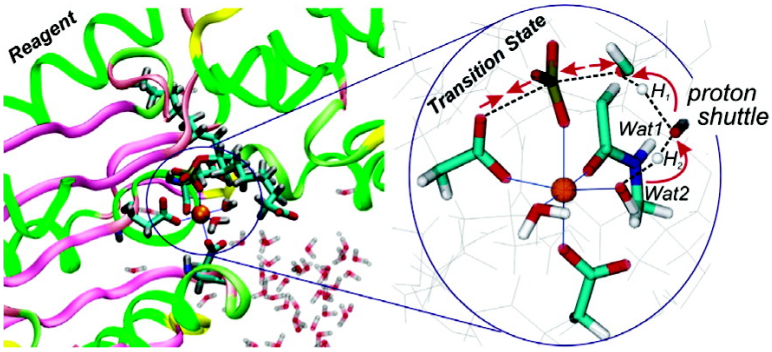

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Computational Study of Phosphatase Activity in Soluble Epoxide Hydrolase: High Efficiency through a Water Bridge Mediated Proton Shuttle

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Recently, a secondary enzymatic activity has been found in the N-terminal domain of the soluble epoxide hydrolase[1] (sEH), an essential ubiquitous enzyme involved in the process of detoxification in living cells.[2] The localized high sEH's presence in mammalian livers, as well as in kidney and vascular tissues,[3a] makes sEH a promising target for hypertension and acute respiratory syndrome treatment.[3b,c]

The novel magnesium-dependent sEH's phosphatase activity transforms phosphorylated hydroxy lipids to their corresponding alcohol/diol products:

$$R{-}O{-}PO_3^{2-} + H_2O \xrightarrow{sEH} R{-}OH + HO{-}PO_3^{2-}$$

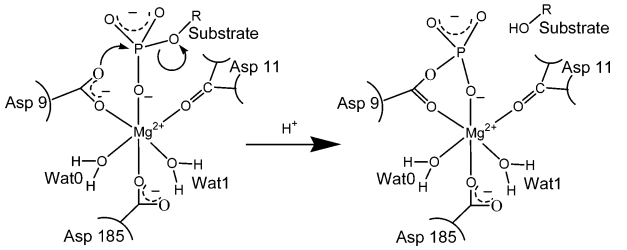
Hence, this finding has opened a new branch of fatty acid metabolism with potential signaling function, also providing a further site for drug targeting.

On the basis of the high homology of the sEH's N-terminal domain with the haloacid dehalogenase (HAD) family members,[4a] along with a recent sEH crystal structure[4b] revealing Mg^{2+} and $HO{-}PO_3^{2-}$ in the N-terminal catalytic site, a two-step reaction mechanism has been proposed:[4b] (1) nucleophilic attack at the phosphate group of the phosphoester substrate by Asp9; (2) hydrolysis of the phosphoenzyme intermediate via a nucleophilic attack of a water molecule at the phosphorus atom. However, mechanistic details of possible reaction mechanisms and related free energy surface (FES) still have to be pinpointed.

In this context, the phosphoryl-transfer reaction in the first step (Scheme 1) of the catalytic cycle has been carefully investigated here using the well-tested[5a] Car−Parrinello (CP) QM/MM approach.[5b] We started from the Michaelis−Menten complex (i.e., *erythro*-9-10-PHO/sEH complex), based on the recent crystal structure of Gomez et al. (PDB entry code 1VJ5).[4b] First, the substrate/N-terminal solvated system was equilibrated by performing ∼10 ns of classical MD simulation (Amber force field[6]). Then, one snapshot was selected as starting point for CP QM/MM calculations. The QM region includes 45 atoms of the reactive region,[7] while all the remaining (∼31 000 atoms) are treated classically.

In the starting configuration, two water molecules (WAT0 and WAT1, Figure 1A) were coordinated to the Mg^{2+} cation in the catalytic site. During the first picoseconds of unconstrained CP QM/MM dynamics, this catalytic Mg^{2+}-centered coordination was perturbed; WAT1 was slowly pulled out of the coordination shell due to the formation of a H-bond (d5, Figure 1A) with the oxygen of the substrate leaving group (O_1, Figure 1A). Then, the highly solvent-exposed catalytic site allowed a third water molecule (WAT2, Figure 1B) to take the original place of WAT1, with the consequent formation of a WAT1-mediated water bridge (WB; $Mg^{2+}{-}WAT2{-}WAT1{-}O_1$, Figure 1B). This well-organized WB-mediated H-bond network stabilized the Mg^{2+}-centered catalytic site coordination.

Scheme 1. Investigated Mechanism[4b] of Phosphoenzyme Intermediate Formation: Phosphate Ester Hydrolysis through Asp9 Nucleophilic Attack



The resulting structure (Reag, Figure 1B) allowed us to define a FES as a function of the reaction coordinate (RC) determined by the difference between the lengths of breaking and forming bonds (RC = $r1 - r2$, Figure 1B). This RC is the natural and minimal choice ensuring that the two reactants approach each other, while all the other degrees of freedom are left free to move. Constrained CP QM/MM simulations of ∼3−4 ps were performed for RC values from ∼ −2.0 to ∼ +0.2 Å, with intervals of ∼0.2 Å. Monitoring the averaged force acting on the constrained RC, the free energy profile was calculated by thermodynamic integration.[5c]

The shape of the resulting surface presents two minima, indicating the reagent and product (i.e., phosphoenzyme intermediate) complexes and one transition state (TS). The free energy barrier determined is ∼19.1 ± 0.5 kcal/mol, in excellent agreement with experimental data[1b,8] (∼18.7 kcal/mol).

Actually, just before the TS, a spontaneous key event occurs; at RC ≈ −0.5 Å (averaged value validated after a backward study along the same RC), a WB-mediated proton shuttle takes place (Figure 2). As Asp9 closely approaches the phosphorus atom ($r2$ ≈ 2.76 Å), the breaking bond $r1$ rapidly elongates ($r2$ ≈ 2.21 Å), leading to its definitive cleavage. This promotes a rise of the basicity of O_1, hence enabling it to act as proton acceptor and to induce a double proton transfer; (i) first, WAT1 gives one proton to the oxygen O_1, forming a good leaving group ($SUB{-}O_1H$); (ii) then, the instantaneous hydroxyl group grabs a proton from WAT2, re-establishing its water nature (WAT1). Thus, WAT2 is now a hydroxyl group; the relative stability of the OH^- is ensured by its coordination (i.e., electrostatic interaction) to the Mg^{2+} cation. In fact, distance $d1$ decreases its averaged starting value ($d1$ ≈ 2.25 Å) to ≈2.05 Å, evidencing a crucial chelating effect of the metal-bound active site.

This highly concerted one-shot double proton transfer (well visible in Figure 2) preludes a narrow interval (−0.4 ≤ RC ≤ 0 Å), in which the average constraint force is essentially zero, indicating a flat region of the FES where the TS is situated (red area in Figure 2). At RC = −0.4 Å, the breaking of $r1$ leads to a

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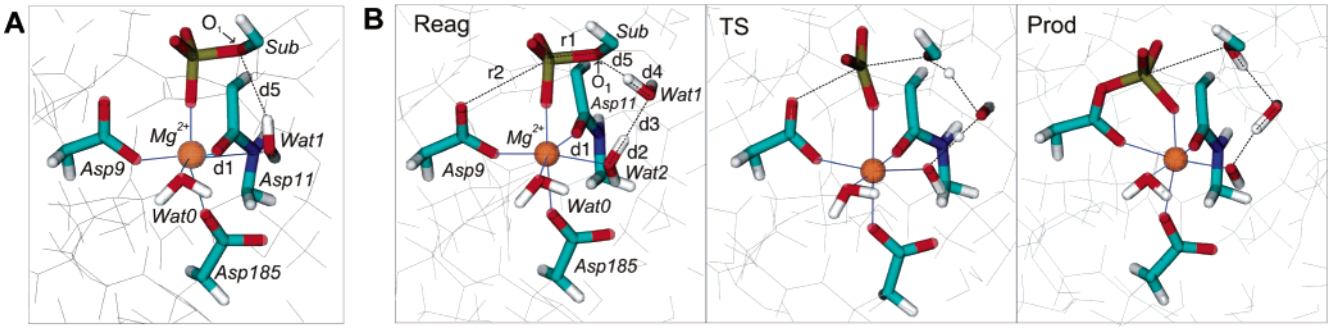

Figure 1. Starting (A) and selected (B) structures of Reag, TS, and Prod states along the CP QM/MM phosphoenzyme intermediate reaction pathway.

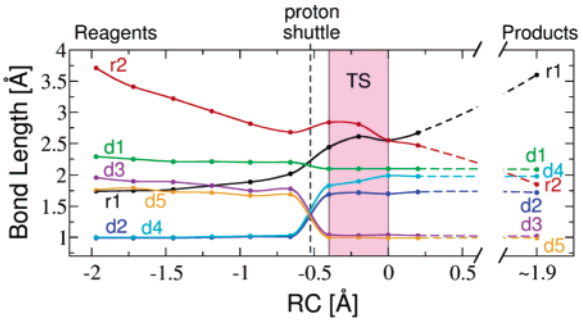

Figure 2. Averaged length of selected bonds and H-bonds along the reaction pathway.

moderate increase of $r2$, then followed by values where, progressively, $r1$ and $r2$ reach the same length ($r1 = r2 \approx 2.56$ Å, RC = 0.0 Å). Thus, the phosphoryl-transfer reaction occurs with a dissociative mechanism, indicated by the presence of the metaphosphate in the S_N2-like TS geometry (Figure 1B), as previously found for this type of reaction in other enzymes.[9] Due to the flat nature of the TS region, the possibility of a pentacoordinated intermediate[10] cannot be definitively ruled out. Nevertheless, both $r1$ and $r2$ do not show values, in the TS region, resembling the phosphorane species. Moreover, if such an intermediate were located in the TS region, it would not change the nature of the result: the energy of an intermediate like that can only be slightly lower (~1−2 kcal/mol) than that of the surrounding TSs. Thus, the presence of such a short-lived intermediate would not significantly affect the reaction profile and, hence, the reaction rate.[11]

At RC = 0.2 Å, the average constraint force changes sign, indicating that the system is evolving toward the products. Hence, upon removal of the constraint, the system falls in the product well in which the phosphoenzyme is fully formed (Figure 1B and Figure 2, RC ≈ 1.9 Å). At this point, the enzyme is ready for the second and final step of the catalytic cycle.

To estimate the catalytic effect of the WB-mediated proton shuttle presented here, we performed the same study of a system in which the original position of WAT1 (i.e., coordinated to the metal) has been maintained applying a restraint on distance $d1$ (Figure 1A). In this case, the phosphoryl-transfer reaction occurs with a similar dissociative pathway, but without any proton transfer involved in the formation of a good leaving group. This mechanism provides a free energy barrier of ~36 ± 1 kcal/mol, which corresponds to a reaction rate ~12 orders slower than the previous WB-mediated reaction pathway thus strongly favored.

In conclusion, here we have presented a computational study of the phosphoenzyme intermediate formation during the novel phosphatase activity of the *bifunctional* sEH enzyme. The phosphoryl-transfer reaction occurs through a dissociative in-line nucleophilic substitution. The calculated free energy fits the experimental value, suggesting that this step might be the rate-determining one of the catalysis, while the TS geometry well resembles crystallographic structures of TS analogues.[12] Importantly, a highly efficient WB-mediated proton shuttle was identified as the crucial event in the reaction. It contributes to speeding up the catalysis by optimizing both the starting reactant complex and the leaving group in the TS. This result shows an elegant way in which the enzyme proficiently performs a concerted multi-event reaction mechanism. Our findings might inspire and be exploited in designing specific TS-based sEH inhibitors.

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Supporting Information Available: Classical MD and QM/MM simulations set up, and TS figure of the second pathway. This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) (a) Cronin, A.; Mowbray, S.; Durk, H.; Homburg, S.; Fleming, I.; Fisslthaler, B.; Oesch, F.; Arand, M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1552. (b) Newman, J. W.; Morisseau, C.; Harris, T. R.; Hammock, B. D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1558.
(2) Hammock, B. D.; Storms, D. H.; Grant, D. F. *Epoxide Hydrolyses, Comprehensive Toxicology*; Guengerich, F. P., Ed.; Pergamon: Oxford, 1997; Vol. 3, pp 283−305.
(3) (a) Zheng, J.; Plopper, C. G.; Lakritz, J.; Storms, D. H.; Hammock, B. D. *Am. J. Respir. Cell. Mol. Biol.* **2001**, *25*, 434. (b) Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K.; Zeldin, D. C.; Laio, J. K. *Science* **1999**, *285*, 1276. (c) Moghaddan, M. F.; Grant, D. F.; Cheek, J. M.; Greene, J. F.; Williamson, K. C.; Hammock, B. D. *Nat. Med.* **1997**, *3*, 652.
(4) (a) Morais, M. C.; Zhang, W.; Baker, A. S.; Zhang, G.; Dunaway-Mariano, D.; Allen, K. N. *Biochemistry* **2000**, *39*, 10385. (b) Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. *Biochemistry* **2004**, *43*, 4716.
(5) (a) Carloni, P.; Rothlisberger, U.; Parrinello, M. *Acc. Chem. Res.* **2002**, *35*, 455. (b) Laio, A.; VandeVondele, J.; Rothlisberger, U. *J. Chem. Phys.* **2002**, *116*, 6941. (c) Ciccotti, G.; Ferrario, M.; Hynes, J. T.; Kapral, R. *Chem. Phys.* **1989**, *129*, 241.
(6) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
(7) The QM region includes the Mg^{2+} cation plus its six coordinated ligands, namely, the side chains of Asp9 and Asp185, the backbone of Asp11, two/three water molecules and the phosphate group of the substrate. Figure 1 shows the QM atoms highlighted.
(8) The experimental reaction barrier was calculated according to the enzymatic reaction theory, using the $k_{cat} = 0.116\ s^{-1}$ reported for the *erythro*-PHO hydrolysis in ref 1b.
(9) (a) Cavalli, A.; De Vivo, M.; Recanatini, M. *Chem. Commun.* **2003**, 1308. (b) Bernardi, F.; Bottoni, A.; De Vivo, M.; Garavelli, M.; Keserü, G.; Náray-Szabó, G. *Chem. Phys. Lett.* **2002**, *362*, 1.
(10) (a) Lahiri, D. S.; Zhang, G.; Dunaway-Mariano, D.; Allen, N. K. *Science* **2003**, *299*, 2067. (b) Allen, N. K.; Dunaway-Mariano, D. *Trends Biochem. Sci.* **2004**, *29*, 495. (c) Tremblay, L. W.; Zhang, G.; Dai, J.; Dunaway-Mariano, D.; Allen, N. K. *J. Am. Chem. Soc.* **2005**, *117*, 5179.
(11) Florián, J.; Warshel, A. *J. Phys. Chem. B* **1998**, *102*, 719.
(12) (a) Schlichting, I.; Reinstein, J. *Nat. Struct. Biol.* **1999**, *6*, 721. (b) Schlichting, I.; Reinstein, J. *Biochemistry* **1997**, *36*, 9290.

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