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SER-HIS-ASP catalytic triad in model non-aqueous solvent environment: A computational study

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Abstract—Emerging new properties and applications of enzymes in organic solvents and ionic liquids are unabating. By applying a combined Quantum Mechanics/Continuum Mechanics computation on a prototypical catalytic triad serine-histidine-aspartate (SER-HIS-ASP) interacting with ethanol or acetonitrile molecules, the major difference between protic and aprotic solvents in effecting transition-state stabilization has been analyzed. Moderately polar aprotic solvent acetonitrile is predicted to be unable to stabilize the transition state in replacing the role of the oxyanion-hole environment, whereas protic ethanol solvent molecules of similar polarity to acetonitrile are adequate in re-gaining the enzymatic activities.

Surging interests in the use of enzymes in non-aqueous solvents as opposed to the presumed native aqueous medium have recently been driven by the emerging new properties and applications that enzymes exhibit in organic solvents.[1–6] Here we attempt to complement the voluminous experimental data in this area by applying a combined Quantum Mechanics/Continuum Mechanics (QM/CM) computation on a simplified model of a representative biocatalytic subsystem—catalytic triad serine-histidine-aspartate (SER-HIS-ASP) interacting with ethanol or acetonitrile (ACN) molecules. SER-HIS-ASP catalytic triad, commonly found in serine proteases, is responsible for the cleavage of peptide bonds through acylation in aqueous solution which is a two-step reaction presumably. The hydroxyl oxygen of the SER side chain ($O1_{SER}$) initiates a nucleophilic attack on the peptidic carbonyl carbon of substrate in forming a tetrahedral oxyanion intermediate while the proton on the same hydroxyl oxygen is migrating to the imidazole nitrogen of the neighboring HIS side chain (Fig. 1). This is followed by the hydrolysis of the peptide bond of the substrate. Some studies have strongly suggested that the side chain of the spatially neighboring protein residues (the 'oxyanion-hole' environment[7]) is instrumental in stabilizing the transition state and the subsequent intermediate.[8,9] Chang et al. reported that medium effect is also important in that regard.[10] This study in particular investigates the plausibility of explicit solvent molecules other than water molecules proximal to the transition states in acting as the oxyanion-hole environment.

Experimentally, serine proteases are generally fairly active in many neat organic or aquo-organic solvent media with respect to synthetic reactions (e.g., esterification, trans-esterification) but with plummeted hydrolytic activities.[1–6] However, one must be cautious in comparing enzymatic reactions in different organic solvents as the thermodynamic activities of the substrates or even the enzyme itself are either not well or difficult to be defined due to varying solubilities.[13] If possible, k_{cat}/K_m should be used for consistent comparisons in various solvents. In addition, the issue of enzyme flexibility in relation to the enantioselectivities of specific and non-specific substrates must be considered also.[13] A recent report revealed that in aquo-organic solvents of 95% organic (ethanol, 1,4-dioxane, or acetonitrile) content, serine protease exhibited comparable (α-chymotrypsin) or even higher (trypsin) residual hydrolytic activities toward specific substrates compared with those measured in water.[14] The results were meaningfully correlated with the secondary structure contents of the enzymes.

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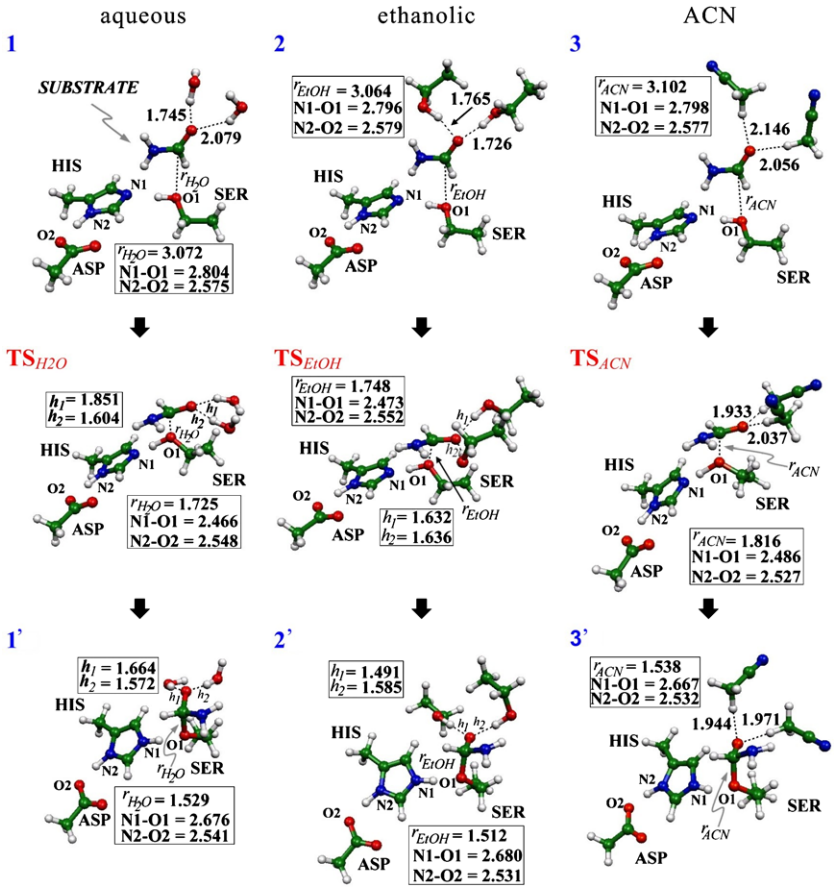

Figure 1. Optimized structures of species involved in the acylation at the HF/3-21G(d) level at the initial (top), transition (middle), and intermediate (bottom) states in the three different solvent environments (left to right: aqueous, ethanolic, ACN). The relevant distances are labeled and indicated in Å. The illustrations are in ball-and-stick (green, carbon; white, hydrogen; blue, nitrogen; red, oxygen).

Unfortunately, the residual hydrolytic activities were only reported in terms of apparent K_m rather than the specific constant (k_{cat}/K_m).

Computational studies on the acylation catalyzed by catalytic triad so far have been exclusively carried out in either gas phase or aqueous medium.[11,12] By investigating the potential energy surface (PES) of the initial acylation step (leading to the acyl-enzyme complex) catalyzed by the SER-HIS-ASP catalytic triad, which is the common rate-determining step in either peptide-bond hydrolysis, esterification, or even transesterification, a clearer picture can be provided for the underlying mechanism of the so-called non-aqueous enzymology in neat organic or aquo-organic solvents. To be specific, the computation here is only directly relevant to the discussion of k_{cat}, which should be complementary to the experimental studies providing K_m information[14] in dissecting the trend of k_{cat}/K_m. Ab initio calculations have previously been performed by others on the QM catalytic triad subsystem, with the rest of the enzyme system represented by capping terminal methyl groups.[12] In this study, the structures of the model reactant, transition state, as well as the tetrahedral intermediate were explicitly compared in model ethanol or acetonitrile environment, and to the control in water.

Two water molecules and a formamide molecule (a minimal peptidic bond unit) were used to simulate the oxyanion hole and the substrate, respectively, as in Nemukhin;[12] while these water molecules were replaced by two ethanol or two ACN molecules in the two non-aqueous counterparts being modeled here. The initial geometry of the catalytic triad in the aqueous system is based on the crystal structure reported at 0.98 Å atomic resolution.[15] Thus the QM subsystem includes totally six species (Fig. 1) which was then optimized at the HF/3–21G(d) level without constraints using Gaussian98.[16] Various initial orientations of the two explicit solvent molecules have been tested and results discussed hereafter are based on the most optimized configurations. The distance between $O1_{SER}$ and the carbonyl carbon of the formamide substrate was then selected as the reaction coordinate and iterative constrained optimizations were carried out at different points along this fixed coordinate in order to obtain the pseudo-minimum-energy path. Full optimization and frequency analysis at the same computational level were extended to the initial, transition, and final points along the chosen reaction coordinate of the systems subsequently. Transition structures are further confirmed by intrinsic reaction coordinate calculations. Based on these optimized structures, the energies of the systems were

recalculated at the B3LYP/6-31+G(d,p) level in continuum solvent by using Jaguar[17] based on the linearized Poisson–Boltzmann formulation by setting the dielectric constant 80.37 for water, 25.30 for ethanol, and 36.64 for ACN.[18] The electrostatic potential used for the fitting of atomic charges was computed on a grid with grid points centered on each atomic nucleus based on self-consistent reaction field model (tolerance set at 5×10^{-5} hartree).

The optimized structures are displayed in Figure 1, and their energies calculated at the B3LYP/6-31+G(d,p) level are summarized (Table 1). The acylation energy profiles along the fixed reaction coordinates are compared by superposition (Fig. 2). The energy barrier for the acylation of the water system is found to be 26.8 kcal mol^{-1}, which is slightly higher than the corresponding value in the ethanol case (25.7 kcal mol^{-1}). These comparable results suggest that the reaction rates of acylation are similar in these two media.[14] Initially, the distance between the HIS imidazole nitrogen and $O1_{SER}$ (N1-O1) is 2.804 Å while the distance between the other HIS imidazole nitrogen and the ASP carboxyl oxygen (N2–O2) is 2.575 Å in **1** of the water system (Fig. 1). The starting reaction coordinate r_{H_2O} in **1** is 3.072 Å which is close to the corresponding value in the ethanol system. In the transition structure $\mathbf{TS}_{H_2O}$, the distance r_{H_2O} is shortened to 1.725 Å while the N1–O1 and N2–O2 distances concomitantly decreased to 2.466 and 2.548 Å (due to the stronger hydrogen bondings between the amino acid residues inside the catalytic triad). Similar phenomenon can be observed in the transition structure of the ethanol case. In passing the transition state, the energy of the system declines and a stable tetrahedral oxyanion intermediate is resulted. The reaction coordinates of the intermediates are further shortened to 1.529 Å (**1′**). The N2–O2 distances in both solvent media stay unchanged essentially while the N1–O1 ones bounce back close to the initial values along with the complete proton transfers.

In contrast, there is no clear transition extremum found in the PES of the ACN system (Fig. 2). Although the

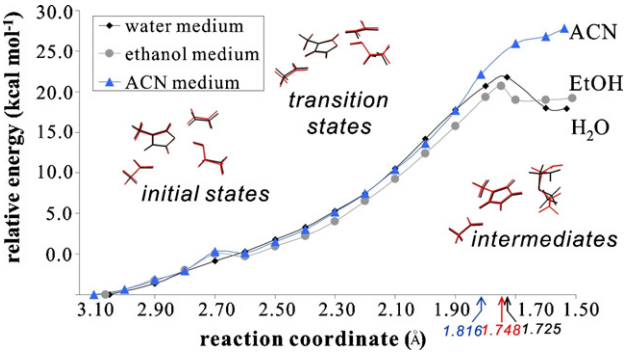

Figure 2. Minimum-energy path for the acylation of the three systems calculated at the B3LYP/6-31G+(d,p) level. The positions of the transition states are indicated on the *x*-axis (blue, ACN; red, EtOH; black, water). The structures of the initial, transition, and intermediate states of the water (black) and ethanol (red) systems are superimposed for illustration (the solvent molecules are ignored for clarity).

presumed transition state (at r_{ACN} = 1.816 Å) and tetrahedral intermediate (at r_{ACN} = 1.538 Å) are able to be located in the optimization level, the energy of the system increases monotonically in the continuum calculation as the reaction coordinate shortened. However, the N1–O1 and N2–O2 distances are close to the water counterpart showing that the structure of the catalytic triad is similar in both media. In $\mathbf{TS}_{ACN}$, the distance r_{ACN} calculated is 1.816 Å which deviates significantly from the corresponding value 1.725 Å in the water system. Although the r_{ACN} (1.538 Å), N1–O1 (2.667 Å), and N2–O2 (2.532 Å) distances in the tetrahedral intermediate (**3′**) are close to the corresponding values (1.529, 2.676, and 2.541 Å, respectively) in the water system, the solution energy obtained in the continuum calculation is not on a minimum point.

The results obtained here illustrate the critical role of the immediate vicinity of the transition states. Moderate to strong hydrogen bondings[19] between the two explicit water/ethanol molecules and the carbonyl oxygen of the formamide is formed throughout the entire process.

Table 1. Total (*E*) and relative (ΔE) energies of the catalytic systems in this study both in the gas and solution continuum phases calculated at the B3LYP/6-31+G(d,p) level

System[a]	The triad + 2 solvent molecules + gas phase environment		The triad + 2 solvent molecules + dielectric continuum solvent	
	E_{gas} (hartrees)	ΔE_{gas} (kcal mol^{-1})	E_{soln} (hartrees)	ΔE_{soln} (kcal mol^{-1})
Solvent: 2 water molecules				
1	−972.04293	0.0	−972.13994	0.0
$\mathbf{TS}_{H_2O}$	−971.99857	27.8	−972.09724	26.8
1′	−972.00378	24.6	−972.10348	22.9
Solvent: 2 ethanol molecules				
2	−1129.28551	0.0	−1129.37175	0.0
$\mathbf{TS}_{EtOH}$	−1129.24434	25.8	−1129.33078	25.7
2′	−1129.24847	23.2	−1129.33318	24.2
Solvent: 2 acetonitrile molecules				
3	−1084.69471	0.0	−1084.78946	0.0
$\mathbf{TS}_{ACN}$	−1084.65457	25.2	−1084.74619	27.2
3′	−1084.64966	28.3	−1084.73727	32.7

[a] See Figure 1 for the system configurations.

Their distances decrease from 2.079 and 1.745 Å (in **1**) to 1.851 and 1.604 Å (in $\mathbf{TS}_{H_2O}$), and finally to 1.664 and 1.572 Å in **1′**, respectively (Fig. 1). Comparable trend but with slightly stronger hydrogen-bonding strength is found in the ethanol case. Only weak interactions, however, are predicted between the ACN molecules and the formamide substrate in **3** (Fig. 1). This result suggests that the interaction formed between ACN and formamide is not strong enough, as expected, for stabilizing the transition state and the oxyanion intermediate.

Interestingly, from Burt's study,[12] no reaction was warranted either in the gas phase or in a dielectric solvent continuum alone without the two explicit solvent water molecules being included. This strongly implies the importance of the interplay between protic solvent and peptidic amide hydrogen in forming the charge-stabilizing environment through specific hydrogen bondings for the rate-determining acylation step. In principle, they are not mutually exclusive in a realistic complex and dynamic enzymatic system but indeed especially indispensable in charge-screening due to the low dielectric environment surrounding the protein surface. This point is general in view of the almost omnipresence of charges induced at the transition states in most enzymatic reactions. A slight local structural distortion of the enzyme caused by external factors such as changed solvent nature in non-aqueous biocatalysis will destroy or diminish the natively evolved optimal charge-stabilizing protein environment. This has to be compensated through other, but often much less effective, molecular mechanisms if it cannot be provided by aprotic solvent molecules such as ACN. The current results might as well imply the re-gained or even enhanced enzyme activity observed in some ionic liquids or ionic liquids of the imidazolium-type suspended in organic solvent via the electrostatic stabilization by the positively charged cations (the anions used in these ionic liquids are often so bulky that they cannot be easily fitted into either the active site or the binding pocket).[20–23]

We have been able to demonstrate the major difference between model protic (e.g., water, ethanol) and aprotic (e.g., acetonitrile) solvents, of similar polarity, in effecting transition-state stabilization which is of great significance, especially to those non-aqueous biocatalytic experiments performed under very low water activities. The results reported here serve as a reference for further studies in dissecting the enzymatic role of the protein environment close to the active site.

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References and notes

1. Carrea, G.; Riva, S. *Angew. Chem. Int. Ed.* **2000**, *39*, 2226.
2. Halling, P. J. *Curr. Opin. Chem. Biol.* **2000**, *4*, 74.
3. Kilbanov, A. M. *Nature* **2001**, *409*, 241.
4. Lee, M.-Y.; Dordick, J. S. *Curr. Opin. Biotechnol.* **2002**, *13*, 376.
5. Khmelnitsky, Y. L.; Rich, J. O. *Curr. Opin. Chem. Biol.* **1999**, *3*, 47.
6. Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194.
7. Creighton, T. E. *Proteins Structures and Molecular Properties*, 2nd ed.; W.H. Freeman: New York, 1996, pp. 417.
8. Fuxreiter, M.; Warshel, A. *J. Am. Chem. Soc.* **1998**, *120*, 183.
9. Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943.
10. Chang, T. K.; Chiang, Y.; Guo, H.-X.; Kresge, A. J.; Mathew, L.; Powell, M. F.; Wells, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 8802.
11. Daggett, V.; Schroder, S.; Kollman, P. *J. Am. Chem. Soc.* **1991**, *113*, 8926.
12. Nemukhin, A. V.; Topol, L. A.; Burt, S. K. *Int. J. Quant. Chem.* **2002**, *88*, 34.
13. Broos, J. *Biocat. Biotransform.* **2002**, *20*, 291.
14. Simon, L. M.; Kotorman, M.; Garab, G.; Laczko, I. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1367.
15. Carter, P.; Wells, J. A. *Nature* **1988**, *332*, 564.
16. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Rega, N.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*.
17. *Jaguar* 4.2, Schrödinger, L.L.C., Portland, 2002. <http://www.schrodinger.com>.
18. Smallwood, I. M. *Handbook of Organic Solvent Properties*; Halsted Press: New York, 1996.
19. Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford: New York, 1997.
20. Hudson, E. P.; Eppler, R. K.; Clark, D. S. *Curr. Opin. Biotechnol.* **2005**, *16*, 1.
21. Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.* **2003**, *14*, 432.
22. Garcia, S.; Lourenço, N. M. T.; Lousa, D.; Sequeira, A. F.; Mimoso, P.; Cabral, J. M. S.; Afonso, C. A. M.; Barreiros, S. *Green Chem.* **2004**, *6*, 466.
23. Sheldon, R. A.; Lau, R. M.; Sorgedrager, M. J.; van Rantwijk, F.; Seddon, K. R. *Green Chem.* **2002**, *4*, 147.