Supermolecule density functional calculations suggest a key role for solvent in alkaline hydrolysis of *p*-nitrophenyl phosphate†

Lidong Zhang,[a] Daiqian Xie,*[a] Dingguo Xu[b] and Hua Guo*[b]

Supermolecule density functional theory calculations show that solvent is responsible for the concerted transition state in alkaline hydrolysis of *p*-nitrophenyl phosphate suggested by heavy atom kinetic isotope effects.

p-Nitrophenyl phosphate (pNPP, Fig. 1) serves as a prototype in studying phosphoryl transfer reactions.[1–4] Such reactions play a prominent role in many biological processes such as cell signaling, DNA polymerization, and energy conversion.[5–7] A key question in understanding the catalytic strategies used by various trans-phosphorylation enzymes, such as kinases and phosphatases, is how they stabilize the transition state relative to the corresponding aqueous solution. In this Communication, we address this question by investigating the alkaline hydrolysis mechanism of pNPP using a theoretical approach.

Extensive experimental data have shown that the alkaline hydrolysis of pNPP proceeds *via* a concerted S_N2 mechanism with a loose metaphosphate-like transition state.[1–3] Such a mechanism, which is common among phosphate monoesters, was implicated by earlier linear free-energy studies.[8] More convincing evidence came in the form of heavy atom kinetic isotope effects (KIEs), which are the rate ratios between the native and substituted isotopomers.[4] A large KIE was found for the bridge phenolic O atom,[9] implying significant bond cleavage to the leaving group at the transition state. Although no transition state structure can be deduced from them, these experimental data, along with the activation parameters for this reaction,[10] provided a unique opportunity to gain microscopic insights into the reaction mechanism by comparison with theory.

Many theoretical studies have been carried out on the hydrolysis of alkyl phosphates,[11–21] but to our best knowledge no such work has been reported for pNPP. Although this is rather surprising
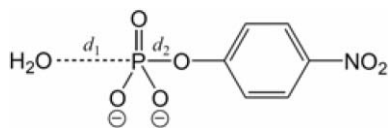

Fig. 1 Water nucleophile and *p*-nitrophenyl phosphate.

[a]*Institute of Theoretical and Computational Chemistry, Laboratory of Mesoscopic Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, People's Republic of China. E-mail: dqxie@nju.edu.cn*
[b]*Department of Chemistry, University of New Mexico, Albuquerque, NM 87131, USA. E-mail: hguo@unm.edu*
† Electronic supplementary information (ESI) available: Coordinates of stationary points along the reaction path obtained by DFT calculations. See DOI: 10.1039/b617946k

given the importance of this reaction, it is well recognized that an accurate theoretical description is difficult because of the anionic nature of such systems, which induces strong solvent effects[15,17,22] Indeed, several recent publications have convincingly demonstrated that gas phase models, even with a posterior continuum-solvent treatment,[23] can be grossly misleading in describing solution reactions involving charged species.[24–28] In the alkaline hydrolysis of amides, for example, the free-energy barrier is completely "solvent induced" since the gas phase model predicts a barrierless reaction path.[29] Thus, a proper treatment of solvation is of paramount importance for these reactions. In many cases, strong solute–solvent interactions such as hydrogen bonds necessitate the explicit inclusion of solvent molecules in theoretical models.

In the current study, our supermolecule models consist of the dianionic pNPP, a hydroxide ion, and n (n = 0, 12, 13, and 14) water molecules. These waters were added in a fashion that maximizes hydrogen bonding with the solute and between solvents. Due to the large number of atoms involved, a mixed basis set was employed. Specifically, the 6-31G(d,p) basis set was used for the nitrophenyl moiety, while the 6-31++G(df,p) and 6-31++G(d,p) basis sets were used for P and the remaining atoms, respectively. The use of diffuse functions is vital to reduce the basis set superposition error (BSSE) for an accurate description of anionic species.[30] Stationary points along the reaction path, including both minima and the transition state, were obtained by full geometry optimization with the B3LYP functional and confirmed by calculations of harmonic frequencies, which were also used to compute the zero-point energy (ZPE) and the thermodynamic properties. The CHELPG charges and natural bond orders were computed to shed further light on the reaction path. The polarized continuum model (PCM)[23] with the 6-31++G(df,p) basis set for all the atoms, a dielectric constant of 78.4, and the UAKS radii was used to account for the bulk solvent surrounding the supermolecule. All calculations were performed with Gaussian 03.[31] Finally, the KIEs at room temperature were obtained using the Bigeleisen–Mayer theory,[32] as implemented in ISOEFF.[33]

In the absence of solvent (n = 0), the isolated pNPP dianion dissociates spontaneously to metaphosphate (PO_3^-) and *p*-nitro-phenoxide (NO_2-C_6H_4-O^-), presumably due to the strong Coulomb repulsion between the two anionic species. This observation highlights the importance of the solvent in stabilizing the anion. In the presence of a few waters, the dissociation is no longer spontaneous and it requires an activation barrier.

When a sufficient number of waters were included, a single transition state (TS) was found, consistent with the established

mechanism for the reaction. As shown in Table 1, the convergence with respect to the number of water molecules is satisfactory for $n > 12$, as the first solvation shell of the phosphate moiety is formed. It is interesting to note that the reaction barrier increases substantially when bulk solvent is included by PCM, underscoring the importance of long-range electrostatic interactions. The activation free energy with 14 water molecules (29.3 kcal mol^{-1}) compares well with the experimental value of 29.5 kcal mol^{-1}.[10]

Fig. 2 displays geometries of the stationary points along the reaction path for the model with 14 explicit water molecules. Interestingly, the nucleophile associated with the reaction path is a neutral water, rather than hydroxide. The latter thus serves as a general base, accepting a proton from the water nucleophile as it attacks the phosphorus center. Attempts have been made to find a reaction path with a hydroxide nucleophile, but were unsuccessful. This observation is particularly interesting because the hydrolysis rate in basic solutions was found to be independent of the pH,[8] implicating a neutral nucleophile.

The transition state in Fig. 2 features a concomitant bond formation between the nucleophilic O and P in pNPP and the bond cleavage to the nitrophenoxide leaving group. The former is evidenced by the shortening of the O–P distance (d_1) from 4.88 Å in RC to 2.65 Å at the saddle point, corresponding to a natural bond order change from 0.001 (RC) to 0.065 (TS), and finally to 0.557 (PC). The latter is manifested by the elongation of the P–O bond (d_2) from 1.76 Å in RC to 2.75 Å at TS, corresponding to a bond order of 0.463 (RC) and 0.060 (TS). The analysis of the reaction coordinate corresponding to the imaginary frequency at TS indicates unmistakeably the involvement of both the addition and elimination coordinates (d_1 and d_2). On the other hand, the bonds between P and three non-bridge O atoms at TS are only slightly shortened from an averaged value of 1.53 Å in RC to 1.51 Å and the transferring phosphoryl group is near planar. Its geometry closely resembles the planar metaphosphate, which has a P–O bond length of 1.50 Å.[28] Furthermore, the benzene ring is distorted at TS to possess increased quinonoid character with alternating single and double bonds, apparently induced by delocalization of the charge brought up by the partially deprotonated nucleophile.

During the reaction, the positive charge on P changes from 0.923e (RC) to 1.148e (TS), and finally to 1.297e (PC). In the meantime, the charge on the phenolic O atom increases modestly as the reaction progresses while that on the nucleophilic oxygen decreases correspondingly. The charges on the non-bridge O atoms do not change much, and are stabilized by hydrogen bonds with nearby solvent molecules, as shown in Fig. 2.

Interestingly, the leaving group is not protonated in PC, reflecting the stability of p-nitrophenoxide (pK_a = 7.14), facilitated presumably by the electron-withdrawing nitro group. As shown in Fig. 2, the phenolic oxygen is hydrogen bonded with two solvent
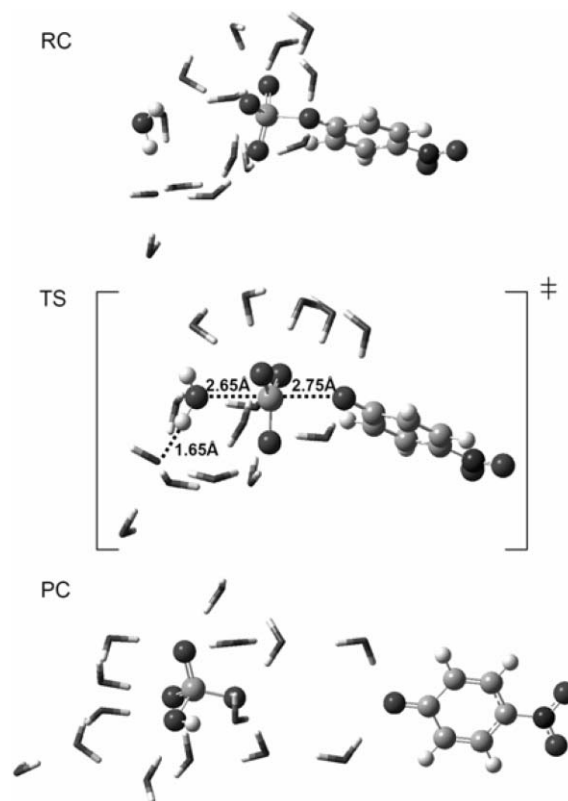

Fig. 2 Geometry of the reactant complex (RC), transition state (TS) and product complex (PC). The partially formed and breaking bonds are represented by dashed lines.

molecules. In PC, a proton in the water nucleophile has been transferred to the hydroxide general base, making it a neutral water.

To make contact with the experimental data,[9] we list in Table 2 the calculated KIEs for the nucleophilic O atom, the bridge O atom, the non-bridge O atoms, and the N atom. The concerted nature of the transition state is reflected by the large and normal KIEs for both the nucleophilic and bridge oxygen atoms, while the participation of the non-bridge oxygen atoms and the nitro group in the reaction coordinate is minimal. This is in overall agreement with the experimental data, although the bridge O seems to play a larger role than indicated by the theoretical model. We attribute the discrepancies to the insufficient number of waters in our model.

The results presented here strongly suggest the intimate involvement of explicit solvent molecules in shaping up the concerted transition state in the hydrolysis of pNPP, providing further evidence for solvent participation in nucleophilic substitution reactions.[22,24–27,29] Although more solvent waters might be needed to provide a complete solvent shell of the entire system, the

Table 1 Energetics of the transition state (TS) and product complex (PC) relative to the reactant complex (RC) (in kcal mol^{-1})

No. waters	$n = 12$	$n = 13$	$n = 14$
TS (ΔE + ZPE)	12.5	22.9	22.9
TS (ΔG_{PCM})	21.1	28.9	29.3
PC (ΔE + ZPE)	−27.0	−26.7	−23.7
PC (ΔG_{PCM})	−18.7	−19.3	−20.7

Table 2 Comparison of experimental and theoretical KIEs

KIEs	$n = 12$	$n = 13$	$n = 14$	Expt.[9]
$^{16/18}O_{nuc}$	1.0088	1.0080	1.0090	—
$^{16/18}O_{bridge}$	1.0165	1.0207	1.0211	1.0189 ± 0.0005
$^{16/18}O_{non-bridge}$ [a]	1.0027	1.0023	1.0029	0.9994 ± 0.0005
$^{14/15}N$	1.0012	1.0012	1.0015	1.0028 ± 0.0002

[a] Averaged over three non-bridge oxygen atoms.

supermolecule model used here is an important step in the right direction. A more accurate characterization of such reactions requires *ab initio* molecular dynamics simulations, as shown by a number of recent studies.[34,35]

In the corresponding enzymatic reactions, the solvent is replaced by hydrogen-bond donating residues such as arginine, which carries a positive charge.[6] These residues differ from the solvent waters in that they are largely immobilized by the protein backbone. The existing experimental evidence indicates that the enzymatic hydrolysis of pNPP typically retains the same mechanism as in the uncatalyzed solution reaction.[3,4] Yet, the origin of the impressive rate enhancement of these enzymes is not entirely clear. Only by comparing the fine details of the transition state for both catalyzed and uncatalyzed reactions using high level theory can we hope to gain further insights into the catalysis.

To summarize, we have for the first time identified the transition state structure for the alkaline hydrolysis of pNPP in aqueous solution by using a supermolecule DFT approach. Reasonable agreement with the experimental activation energy and KIEs has been achieved. Our calculations confirmed that the nucleophile in the reaction is a neutral water, and the hydroxide serves as a general base. The transition state geometry provides microscopic insight into this prototypical solution phase reaction and serves as a reference to analogous reactions catalyzed by enzymes.

Notes and references

1 S. J. Benkovic and K. J. Schray, in *Transition States of Biochemical Processes*, ed. R. D. Gandour, Plenum, New York, 1978.
2 G. R. J. Thatcher and R. Kluger, *Adv. Phys. Org. Chem.*, 1989, **25**, 99.
3 A. C. Hengge, in *Comprehensive Biological Catalysis*, ed. M. Sinnot, Academic Press, San Diego, 1998, Vol. 1.
4 A. C. Hengge, *Acc. Chem. Res.*, 2002, **35**, 105.
5 F. H. Westheimer, *Science*, 1987, **235**, 1173.
6 A. S. Mildvan, *Proteins: Struct., Funct., Genet.*, 1997, **29**, 401.
7 M. Oivanen, S. Kuusela and H. Lonnberg, *Chem. Rev.*, 1998, **98**, 961.
8 A. J. Kirby and A. G. Varvoglis, *J. Am. Chem. Soc.*, 1967, **89**, 415.
9 A. C. Hengge, W. A. Edens and H. Elsing, *J. Am. Chem. Soc.*, 1994, **116**, 5045.
10 A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, 1965, **87**, 3209.
11 C. Lim and M. Karplus, *J. Am. Chem. Soc.*, 1990, **112**, 5872.
12 A. Dejaegere, X. Liang and M. Karplus, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 1763.
13 J. Florian and A. Warshel, *J. Phys. Chem. B*, 1998, **102**, 719.
14 J. Florian and A. Warshel, *J. Am. Chem. Soc.*, 1998, **120**, 11524.
15 C.-H. Hu and T. Brinck, *J. Phys. Chem. A*, 1999, **103**, 5379.
16 J. M. Mercero, P. Barrett, C. W. Lam, J. E. Flower, J. M. Ugalde and L. Pedersen, *J. Comput. Chem.*, 2000, **21**, 43.
17 X. Lopez, A. Dejaegere and M. Karplus, *J. Am. Chem. Soc.*, 2001, **123**, 11755.
18 M. Bianciotto, J.-C. Barthelat and A. Vigroux, *J. Am. Chem. Soc.*, 2002, **124**, 7573.
19 M. Bianciotto, J.-C. Barthelat and A. Vigroux, *J. Phys. Chem. A*, 2002, **106**, 6521.
20 Y.-N. Wang, I. A. Topol, J. R. Collins and S. K. Burt, *J. Am. Chem. Soc.*, 2003, **125**, 13265.
21 M. Klahn, E. Rosta and A. Warshel, *J. Am. Chem. Soc.*, 2006, **128**, 15310.
22 B. A. Gregersen, X. Lopez and D. M. York, *J. Am. Chem. Soc.*, 2004, **126**, 7504.
23 J. Tomasi and M. Persico, *Chem. Rev.*, 1994, **94**, 2027.
24 C. G. Zhan, D. W. Landry and R. L. Ornstein, *J. Am. Chem. Soc.*, 2000, **122**, 2621.
25 J. R. Pliego, *Chem. Phys.*, 2004, **306**, 273.
26 D. Xie, Y. Zhou, D. Xu and H. Guo, *Org. Lett.*, 2005, **7**, 2093.
27 D. Xu, H. Guo, Y. Liu and D. M. York, *J. Phys. Chem. B*, 2005, **109**, 13827.
28 L. Zhang, D. Xie, D. Xu and H. Guo, *J. Phys. Chem. A*, 2005, **109**, 11295.
29 S. J. Weiner, C. Singh and P. A. Kollman, *J. Am. Chem. Soc.*, 1985, **107**, 2219.
30 J.-H. Lii, B. Ma and N. L. Allinger, *J. Comput. Chem.*, 1999, **20**, 1593.
31 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, T. Vreven Jr., K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian 03, Revision A.1 (Gaussian, Inc., Pittsburgh, PA, 2003).
32 J. Bigeleisen and M. G. Mayer, *J. Chem. Phys.*, 1947, **15**, 261.
33 V. Anisimov and P. Paneth, *J. Math. Chem.*, 1999, **26**, 75.
34 M. Cascele, S. Raugei and P. Carloni, *J. Phys. Chem.*, 2004, **108**, 369.
35 J. Blumberger and M. L. Klein, *Chem. Phys. Lett.*, 2006, **422**, 210.