Supporting Information for :

Quantifying the Mechanism of Phosphate Monoester Hydrolysis in Aqueous Solution by Evaluating the Relevant *ab initio* QM/MM Free Energy Surfaces

Nikolay V. Plotnikov[1], B. Ram Prasad[1], Suman Chakrabarty[2], Zhen T. Chu[1],
and Arieh Warshel[1]*

[1]Department of Chemistry, University of Southern California, SGM 418, 3620 McClintock Avenue, Los Angeles, California 90089.

[2]Physical Chemistry Division, National Chemical Laboratory (NCL), Dr. Homi Bhabha Road, Pashan, Pune, Maharashtra, 411 008, India.

I .Implicit solvent calculations

Although the most important part of this work involved QM(ai)/MM MD simulations , we also extended our previous implicit solvent study. As discussed in our previous work,[1] mapping the potential surface of phosphate hydrolysis in solutions is very challenging when done with minimization approaches and implicit solvent models, and in particular in cases when one of the two alternative paths are in close (with the given resolution) regions of the free energy surface of similar heights but with higher energy. Here we tried to improve the results by staying near the regions located in our previous study and performing full TS minimization with the given implicit solvent model (the corresponding results were then verified by MD PMF calculations). Furthermore to reduce the problems associated with using 2-quantum water in the 1W path we performed this study with only one quantum water, thus gaining more stability in the calculations. All the ab initio calculations (with the PCM minimization) were carried out using the Gaussian03 software package.[2] The geometry optimizations were performed in the IEFPCM implicit solvent model[3] using 6-31+G* basis set and the B3LYP hybrid functional.[4] The calculated free energy surfaces were used then to identify the approximate location and energies of key stationary points.[1]

Our previous study involved the study of the hydrolysis of monomethyl pyrophosphate trianion, using implicit solvent models with systematic search but with constrained minimization. The difficulties were compounded by attempting to find the 1W TS in the presence of an additional water molecule. Here we made an additional attempt studying the 1W case with only 1 quantum water and using now the complete PCM minimization option. The corresponding results are compiled in Table 1. The Table shows now a significantly larger difference between the 2W and 1W cases. These results strengthen our conviction that only converging QM(ai)/MM free energy studies, with a careful exploration in the dimensions where it is possible to find a significant barrier, can provide sufficient confidence in the mechanistic results. Thus we conducted the systematic studies described in the main text .

II Additional calculations

Our study also examined the effect of the size of the QM region on the computed energetics. The free energy surfaces were computed for various solute models. The PM3/MM free energy surfaces for MHDP and MDP (see in Figure 5 and Figure 7 of the main text) are given in Figure 6 and Figure 8 respectively. Additionally we also calculated PM3/MM surfaces for MDP with Na^+ (where Mg^{2+} in the model of Figure 7 was replaced by Na^+) and for MTP (with an additional phosphate group added to the model of Figure 7) which are depicted in Figure S1. The computed free energy surfaces suggests that the catalytic hydrolysis of MDP could be utilized as a simple (yet reliable) model for studying the MTP hydrolysis. Here the Mg^{2+} seems to make the associative pathway lower than the dissociative one, which becomes of the similar (Na^{+}) or of the lower height (MHDP) in the absence of Mg^{2+}.

References

(1) Ram Prasad, B.; Plotnikov, N.; Warshel, A.: Addressing open questions about phosphate hydrolysis pathways by careful free energy mapping. *J. Phys. Chem. B* **2013**, *117*, 153-163.

(2) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, J. T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; J. Tomasi; V. Barone, B. M., M. Cossi, G. Scalmani, N. Rega,; G. A. Petersson, H. N., M. Hada, M. Ehara, K. Toyota,; R. Fukuda, J. H., M. Ishida, T. Nakajima, Y. Honda, O. Kitao,; H. Nakai, M. K., X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross,; C. Adamo, J. J., R. Gomperts, R. E. Stratmann, O. Yazyev,; A. J. Austin, R. C., C. Pomelli, J. W. Ochterski, P. Y. Ayala,; K. Morokuma, G. A. V., P. Salvador, J. J. Dannenberg,; V. G. Zakrzewski, S. D., A. D. Daniels, M. C. Strain,; O. Farkas, D. K. M., A. D. Rabuck, K. Raghavachari,; J. B. Foresman, J. V. O., Q. Cui, A. G. Baboul, S. Clifford,; J. Cioslowski, B. B. S., G. Liu, A. Liashenko, P. Piskorz,; I. Komaromi, R. L. M., D. J. Fox, T. Keith, M. A. Al-Laham,; C. Y. Peng, A. N., M. Challacombe, P. M. W. Gill,; B. Johnson, W. C., M. W. Wong, C. Gonzalez, and J. A. Pople: Gaussian 03, Revision C.03. In *Gaussian, Inc., Wallingford CT*, 2004.

(3) Cancès, E.; Mennucci, B.; Tomasi, J.: A new integral equation formalism for the polarizable continuum model: Theoretical background and applications to isotropic and anisotropic dielectrics. *J. Chem. Phys.* **1997**, *107*, 3032-3041.

(4) Becke, A. D.: Density-Functional Thermochemistry .3. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*, 5648-5652.

(5) Štrajbl, M.; Florián, J.; Warshel, A.: Ab Initio Evaluation of the Potential Surface for General Base-Catalyzed Methanolysis of Formamide: A Reference Solution Reaction for Studies of Serine Proteases. *J. Am. Chem. Soc.* **2000**, *122*, 5354-5366.

Table 1. Summary of the results obtained from energy minimization studies with implicit solvent model(a)

System/path & Model[b]	R_2	R_1	$(X_1/X_2)_{TS}$	$\xi = (X_1 - X_2)$	$\Delta E^{\ddagger}$	$\Delta E^{\ddagger}$ + ZPE	$\Delta G^{\ddagger} = (\Delta E^{\ddagger}$ + ZPE-$T\Delta S^{\ddagger})$[c]
$PO_3^{(-)}$ with 1 QM H_2O + ΔG ($R_1 \rightarrow \infty$)[d]							
INT	2.0	--	0.99/2.89	-1.90	0.0 (18.82)[d]	--	--
TS (1W, PT)	1.87	--	1.19/1.32	-0.13	17.19 (36.01)[d]	14.82(33.64)[d]	15.49(34.31)[d]
$PO_3^{(-)}$ with 2 QM H_2O + ΔG ($R_1 \rightarrow \infty$)[d]							
INT	2.0	--	1.00/1.67	-0.67	0.0 (18.13)[d]	--	--
TS (2W, PT)	1.80	--	1.27/1.17	0.10	4.64 (22.77)[d]	1.7(19.83)[d]	2.45(20.58)[d]
MDP with 1 QM H_2O							
RS	3.0	1.71	--	--	0.0	--	--
Asso/Conc. INT	2.0	4.0	0.99/2.89	-1.90	18.32	--	--
Asso/Conc. TS (1W, PT)	1.9	4.0	1.25/1.25	0.0	17.65[f] (35.96)[g]	33.59	34.26
Asso/Conc. TS (1W, PT) (cosmo)[e]	1.9	4.0	1.25/1.25	0.0	17.44[f] (35.76)[g]	33.39	34.06
MDPH with 1 QM H_2O							
Asso/Conc. TS (1W, PT)	1.9	4.0	1.25/1.25	0.0	16.6[f] (34.92)[g]	32.55	33.22
MDP with 2 QM H_2O							
RS	3.0	1.71	--	--	0.0	--	--
Asso/Conc. INT	2.0	4.0	1.01/1.67	-0.66	18.32	--	--
Asso/Conc. TS (2W, PT)	2.0	4.0	1.25/1.25	0.0	5.52[f] (23.84)[g]	20.9	21.65
Asso/Conc. TS (2W, PT) (cosmo)[e]	2.0	4.0	1.25/1.25	0.0	6.90[f] (25.22)[g]	22.28	23.03
MDPH with 2 QM H_2O							
Asso/Conc. TS (2W, PT)	2.0	4.0	1.25/1.25	0.0	3.2[f] (21.52)[g]	18.58	19.33
MDP with 1 QM H_2O							
RS	3.0	1.71	--	--	0.0	--	--
Asso. INT	2.1	1.93	0.98/2.17	-1.19	31.31	--	--
Asso. TS (1W, PT)	2.1	1.9	1.3/1.3	0.0	40.91	38.54	39.21

[a] Energies in kcal/mol.

[b] All the calculations were carried out using IEF-PCM solvent model unless otherwise indicated.

[c] The zero point energy and entropy corrections are evaluated with the harmonic approximation (used in Gaussian) . The zero point correction does not involve tunneling correction and the harmonic entropy calculations are

problematic and are scaled by 0.5 (see ref . [5] for discussion) .

[d] Here the zero energy is taken as that of the energy of MDP when R_1 is taken to 5Å (which is considered as infinite separation).

[e] The minimizations were carried out using the COSMO solvent model.

[f] Here the designatedINT energy is considered as the reference zero energy.

[g] Here the designated RS energy is considered as the reference zero energy.

Figure S1. PM3/MM free energy surfaces for: (A) MTP plus Mg^{2+} plus 16 QM H_2O (B) MDP plus Na^+ plus 16 QM H_2O. The obvious effect of Mg^{2+} is that it seems to change the probability of the associative/dissociative paths, making the associative more likely. Probably it provides stabilization to the associative complex by decreasing the repulsion between the negatively charged leaving terminal phosphate and the residual methyl(di)phosphate Also Na^+ seems to be doing worse than Mg^{2+} in terms of lowering the associative TS1 .