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Hydrolysis Rate Difference between Cyclic and Acyclic Phosphate Esters: Solvation versus Strain[†]

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Phosphorus has a broad role in living systems, and so the reactions of phosphate esters in solution and in enzymes are of great interest.[1] The classic work on the hydrolysis of phosphates by Westheimer and co-workers[2] showed that five-membered-ring compounds (e.g., ethylene phosphate (EP)) are hydrolyzed 10^6–10^8 times faster than acyclic compounds (e.g., dimethyl phosphate, DMP).[3] This difference has been ascribed to ground-state destabilization arising from strain in the cyclic reactant, which is releaved in the trigonal bipyramidal transition state.[2,4] In this communication, we use gas-phase *ab initio* calculations to show that although there is strain in the ground state of the cyclic reactant, it does not contribute to the rate acceleration. Further, an estimate of solvation effects from a continuum model suggests that most of the rate acceleration of the cyclic versus the acyclic phosphates arises from differential solvation of the transition states.

The structure and energies of the reactants and transition states in the gas phase were obtained by full optimization at the HF/3-21+G(d,d) and MP2/6-31+G* levels using Gaussian 90.[5] Previous calculations on phosphates and phosphoranes anions have shown that both polarization and diffuse functions are needed to obtain reliable results.[6–8] The results for the activation parameters are summarized in Table I. It can be seen that the calculated values for the acyclic phosphate (DMP) and the cyclic phosphate (EP) are very similar. Since energy strain should be present in gas-phase calculations, this result does not support the standard model for the difference of approximately 10 kcal/mol in the activation free energy between the cyclic and the acyclic compound; solution measurements have shown that the difference is mainly enthalpic.[4] The *ab initio* results do confirm the structural and thermodynamic data that indicate that there is strain in the ground state of the cyclic esters (Table II). X-ray crystallographic studies have shown that both the O–P–O and P–O–C angles are significantly smaller in five-membered ring phosphate esters than in other phosphate esters.[9–12] In accord with experiment, the theoretical O–P–O angle is about 6° smaller for the cyclic reactants.[13] The calculations also support the hypothesis that the O–P–O strain is relieved in the transition state, where the O–P–O angles are the same for DMP and EP; the P–O–C angle remains strained in the cyclic transition state. To estimate the strain energy, theoretical values of the total energy of ethylene, dimethyl, trimethylene, and methyl ethyl phosphate are compared, that is,

$$E_{\text{strain}} = (E_{\text{EP}} - E_{\text{DMP}}) - (E_{\text{TMP}} - E_{\text{MEP}}) \qquad (1)$$

This comparison is similar to that used in studies of the strain energy of cyclopentane[15] (trimethylene phosphate is used as an unstrained cyclic phosphate);[2,14] extraneous contributions cancel in the double difference in eq 1. The *ab initio* results for the strain are 2.2 kcal/mol at the HF/3-21+G(d,d) level and 3.8 kcal/mol at the MP2/6-31+G* level with fully optimized geometries; if vibration contributions are included, the strain enthalpy is 0.5 kcal/mol less. Calorimetric measurements of the enthalpy of hydrolysis of cyclic phosphates yield values that are 3.5–6 kcal/mol larger than those for the hydrolysis of related acyclic compounds.[16] This difference is identified as strain energy with the assumption[16] that other factors cancel. Considering the uncertainties in the experimental values, e.g., the hydrolysis of an acyclic ester yields two products and the hydrolysis of a cyclic ester yields only one product, the agreement with the *ab initio* results is satisfactory.

Table I. Gas-Phase Activation Parameters for the Basic Hydrolysis of Dimethyl Phosphate (DMP) and Ethylene Phosphate (EP) in kcal/mol[a]

	energy		enthalpy		free energy	
	DMP	EP	DMP	EP	DMP	EP
HF/3-21+G(d,d)	95.4	94.4	95.5	94.3	106.5	103.4
MP2/6-31+G*	84.7	85.0	84.8	85.2	95.6	94.0

[a] The values reported for the energy are the differences between the *ab initio* values for the reactants and transition states; in the enthalpies and free energies, all thermal contributions are included.[17,27]

Table II. Angles (in deg) Characteristic of Five-Membered-Ring and Other Phosphate Esters[a]

angle	acyclic and six-membered ring	five-membered ring
	Experimental Reactants	
O–P–O (O ester)	102–108	96–98
P–O–C	≈120	≈112
	Ab Initio Reactants[b]	
O–P–O (O ester)		
anion	98–100	93
neutral	106	96
P–O–C	116–119	111–113
	Ab Initio Transition States[b]	
O–P–O (O ester)	88	87
P–O–C	120	111–114

[a] The compounds used in the comparison are dimethyl phosphate (neutral and anionic), ethylene phosphate (neutral and anionic), trimethyl phosphate (anionic), methyl ethyl phosphate (anionic), and the dianionic phosphorane transition states for the hydrolysis of dimethyl and ethylene phosphate. [b] Results are for HF/3-21+G(d,d) and MP2/6-31G* calculations.

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(1) Westheimer, F. H. *Science* **1987**, *235*, 1173.

(2) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.

(3) Haake, P. C.; Westheimer, F. H. *J. Am. Chem. Soc.* **1961**, *83*, 1102.

(4) Kluger, R.; Taylor, S. D. *J. Am. Chem. Soc.* **1990**, *112*, 6669.

(5) (a) Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schlegel, H. B.; Raghavachari, K.; Robb, M.; Binkley, J. S.; Gonzalez, C.; Defrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, L. R.; Stewart, J. J. P.; Topiol, S.; Pople, J. A. *Gaussian 90*, Revision I; Gaussian, Inc.: Pittsburgh, PA, 1990. (b) The notation 3-21+G(d,d) is used to designate a split-valence basis set (3-21G) augmented with diffuse (sp) and polarization (d) functions. These sp and d functions are the same as those used in the 6-31+G* basis set, and they are added to all non-hydrogen atoms. This basis set has the same number of basis functions as the more conventional 6-31+G* basis but a reduced number of Gaussian primitives, which decreases the time required for geometry optimization; see: Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley and Sons: New York, 1986.

(6) Magnusson, E. *J. Comput. Chem.* **1984**, *5*, 612.

(7) Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872.

(8) Dejaegere, A.; Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1991**, *113*, 4353.

(9) Gerlt, J. A.; Westheimer, F. H.; Sturtevant, J. M. *J. Biol. Chem.* **1975**, *250*, 5059.

(10) Haque, M. U.; Caughlan, C. N.; Moats, W. L. *J. Org. Chem.* **1970**, *35*, 1446.

(11) Chiu, Y. H.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1969**, *91*, 4150.

(12) Coulter, C. L. *J. Am. Chem. Soc.* **1975**, *97*, 4084.

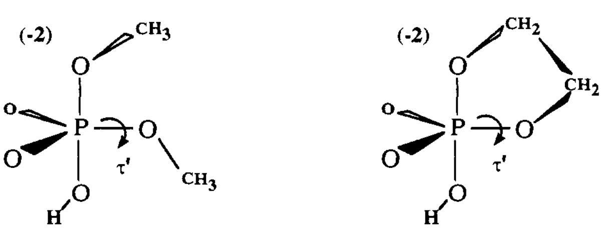
(13) The difference between calculated and experimental angles may come from the fact that the reactants considered here are anions, whereas the experimental values refer to phosphates that are protonated or crystallized with a counterion. For protonated dimethyl and ethylene phosphate, the calculated angles are within the range of the experimental angles.

(14) Wall, R. E. Ph.D. Thesis, Harvard University, 1960.

(15) Allinger, N. A.; Tribble, M. T.; Miller, M. A.; Wertz, D. H. *J. Am. Chem. Soc.* **1971**, *93*, 1637.

(16) Taylor, S. D.; Kluger, R. *J. Am. Chem. Soc.* **1992**, *114*, 3067.

0002-7863/93/1515-5316$04.00/0



DMP Transition State EP Transition State

Figure 1. Transition state for the basic hydrolysis of DMP^- and EP^- showing the dihedral angle τ' involved in the reaction (see text).

Since the *ab initio* results confirm the existence of strain in the five-membered-ring reactants and yield a negligible difference in the calculated activation enthalpies of the cyclic and acyclic compounds, there must be a destabilization of the cyclic transition state that cancels the ground-state destabilization. This arises from the impossibility for the cyclic phosphate to adopt the most favored conformation in the transition state resulting from attack by OH^-. Calculations of the reaction profile[8,17,18] have shown that attack of OH^- on DMP has a transition state with a trans orientation of the equatorial methoxy group ($\tau' = 137°$, see Figure 1). The energetic cost of attack in an unfavorable conformation is 5–6 kcal/mol.[17] In the EP transition state, the values of τ' is 37.3° due to the lack of conformational flexibility in the ring. This leads to a destabilization which compensates approximately for the ground-state destabilization of the cyclic reactant.

Aqueous solvation is expected to have a large effect on the activation energy since a doubly charged ion is formed from two singly charged species.[8] To obtain an estimate of the solvation effects, we have used a finite difference Poisson–Boltzmann continuum model.[19,20] Calculations based on a spherical cavity with an effective radius have been used for the study of the solvent effect on the energies of hydrolysis of phosphoric and carboxylic anhydrides.[21] The method employed here avoids the need for introducing a spherical cavity by using the van der Waals envelope for the molecule.[19,20,22] It has been shown that such calculations may provide an accurate description of solvation when electrostatic effects are dominant.[23,24] The results are presented in Table III. The partial atomic charges are based on Mulliken populations from HF/6-31G* single-point calculations at the 3-21+G(d,d) geometries. Mulliken charges calculated at the 6-31G* level generally show a good correlation with charges determined using more sophisticated methods. They have been used for the interpretation of the solvent effect on reaction profiles.[25] The values of the solvation free energies of the various ions are large, as expected. For DMP, they reduce the activation free energy from 95 kcal/mol to 38 kcal/mol; this is in reasonable agreement with the experimental estimate of 32 kcal/mol.[3,26] More important, the solvation contribution leads to a significantly larger lowering for the cyclic than the acyclic system so that the resulting difference is in agreement with the experimental difference. Other contributions to the solvation free energy (e.g., van der Waals) terms are expected to cancel to a large extent when the two systems are compared.

Table III. Estimated Activation Free Energies in Water for the Hydrolysis of Dimethyl and Ethylene Phosphate in Units of kcal/mol[a]

Free Energies of Solvation[b]

DMP	EP	OH	DMP-TS	EP-TS
−71.4	−74.0	−94.7	−224.0	−233.2

Activation Free Energies in Solution[c]

	DMP	EP	Δ
HF	48.6	39.0	+9.6
MP2	37.7	29.6	+8.1

[a] The reactions are $DMP^- + OH^- \rightarrow TS^{2-}$ and $EP^- + OH^- \rightarrow TS^{2-}$; only electrostatic contributions to the solvation free energy of reactants and products are included. [b] See refs 19, 20, and 28. [c] The activation free energies are estimated by combining the solvation free energies with the gas-phase values.

The results presented here provide evidence that solvation is important in determining the relative rates of reaction of cyclic and acyclic phosphate esters. The small difference in the activation enthalpies computed for the cyclic and acyclic compounds in the gas phase appears to invalidate the strain model for these reactions. Further calculations and experiments are needed to test this interpretation.

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(17) Dejaegere, A.; Karplus, M., manuscript in preparation.

(18) In related work on CH_3O^- attack on DMP, Taira *et al.* have shown a corresponding τ' dependence. See: Uchimaru, T.; Tanabe, K.; Nishikawa, S.; Taira, K. *J. Am. Chem. Soc.* **1991**, *113*, 4351. Taira, K.; Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Nishikawa, S. *Nucleic Acids Res.* **1991**, *19*, 2747.

(19) Bashford, D.; Karplus, M. *Biochemistry* **1990**, *29*, 10219.

(20) Lim, C.; Bashford, D.; Karplus, M. *J. Phys. Chem.* **1991**, *95*, 5610.

(21) Hayes, D. M.; Kenyon, G. L.; Kollman, P. A. *J. Am. Chem. Soc.* **1978**, *100*, 4331.

(22) Gilson, M.; Sharp, K.; Honig, B. *J. Comput. Chem.* **1988**, *9*, 327.

(23) Arald, J.-C.; Nicholls, A.; Sharp, K.; Honig, B.; Tempczyk, A.; Hendrickson, T. F.; Still, W. C. *J. Am. Chem. Soc.* **1991**, *113*, 1454.

(24) Mohan, V.; Davis, M. E.; McCammon, J. A.; Pettitt, B. M. *J. Phys. Chem.* **1992**, *96*, 6428.

(25) Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 7430.

(26) From the rate constants at 115 and 125 °C, the entropy contribution is estimated to be about 4 kcal/mol at 25 °C; see: Kumamoto, J.; Cox, J. R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 4858.

(27) The enthalpy was obtained as the sum of the HF or MP2 energy, the zero-point vibrational energy (ZPVE), the vibrational correction to the ZPVE at 298.15 K; the rotational and translational energies, and the $P\delta V$ terms. The zero-point vibrational energy and the thermal vibrational energy were calculated in the rigid rotor–harmonic oscillator approximation by statistical thermodynamics.[29] The rotational and translational energies were treated classically as $^1/_2RT$ per degree of freedom. The 3-21+G(d,d) geometries and analytical forces constants were used to calculate the vibrational frequencies. These frequencies were used unscaled to compute the SCF enthalpy but were scaled by 0.89 to estimate the MP2 barrier; the free energies included translational, rotational and vibrational entropy terms.

(28) The linearized Poisson–Boltzmann equations are solved on a cubic lattice of 80-Å length centered on the phosphorus. The initial lattice spacing is 1.0 Å; calculations around the solute are refined using a smaller grids with 0.125 Å spacing and 81 lattice points. The volume occupied by the solute is determined using a spherical probe of 1.4 Å rolling over the surface of the solute. The values of the dielectric constant are 2 for the solute and 80 for the solvent. The radii[30,31] used to define the solute shape are phosphorus, 2.15; phosphoryl oxygen, 1.70; ester oxygen, 1.77; carbon, 1.80; aliphatic proton, 1.468; hydroxyl oxygen, 1.796; and hydroxyl proton, 0.73 Å. Test calculations have demonstrated that the relative values of the solvation free energies of the transition state and reactants are not affected by reasonable variations in the parameters, such as the grid spacing, the solute dielectric constant, and the atomic radii.

(29) McQuarrie, D. A. *Statistical Mechanics*; Harper & Row: New York, 1976.

(30) MacKerell, A. D.; Wiorkiewicz, J.; Karplus, M., to be submitted for publication.

(31) Madura, J. D.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1986**, *108*, 2517.