Do Pentacoordinate Oxyphosphorane Intermediates Always Exist?

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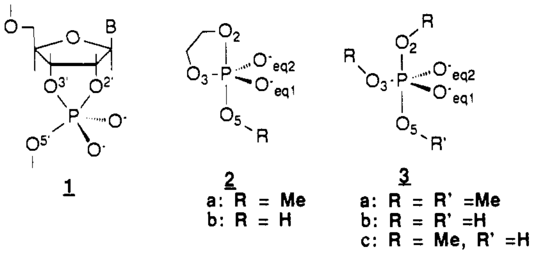
A pentacoordinate oxyphosphorane species is a common intermediate or transition state during phosphoryl-transfer reactions.[1,2] Hydrolysis of RNAs proceeds via the cyclic oxyphosphorane species **1**.[3,4] Recently, Karplus's group[5,6] and ours[7-11] carried out ab initio investigations on the reaction profile of phosphoryl-transfer reactions using cyclic **2** and acyclic **3** oxyphosphoranes as model compounds for these reactions (Scheme I). These studies indicated that, although *dianionic* intermediates **2** and **3**, with potential well depths that are at most of the order of k_BT, do not exist along the gas-phase reaction coordinate,[5,6,7b,c,8,9] the corresponding *monoanionic* form designated **2a** can be located as a stable intermediate on the potential surface.[10]

During the acid-catalyzed hydrolysis of hydrogen ethylene phosphate and methyl ethylene phosphate in ^{18}O-labeled water, the labeled oxygen can be incorporated into the unreacted ester.[12,13] In addition, significant phosphoryl migration from the 3'- to the 2'-hydroxyl group of the ribonucleotide takes place under acidic conditions.[3,14,15] These results suggest the existence of a pentacoordinate intermediate that can undergo pseudorotation in acidic media. By contrast, base-catalyzed hydrolysis is not accompanied by ^{18}O exchange,[12] and no phosphoryl migration is apparent.[3,14,15] In accord with the calculations, the latter results can be rationalized by assuming the nonexistence of pentacoordinate intermediates in basic media.[5,6,8-10] However, we started to wonder whether the conclusions from gas-phase studies about the nonexistence of a pentacoordinate intermediate in the base-catalyzed hydrolysis of phosphate could really be applied to studies in solution.[9] Indeed, nonexistence of *dianionic* intermediates **2** and **3** could be explained by the possibility that they lack the ability to delocalize two negative charges stably in the gas phase. In accord with this hypothesis, the smallest *dianionic* oxyphosphorane **3b** turned out to be the least stable molecule and gave no indication of a well in the energy potential.[9] This result contrasts with results for **3a**[8] and **3c**[6] which have wells with a depth of the order of k_BT. If our hypothesis is correct, any *dianionic* oxyphosphorane should be able to exist as an intermediate once it has been solvated.[16]

Scheme I



1

2
a: R = Me
b: R = H

3
a: R = R' =Me
b: R = R' =H
c: R = Me, R' =H

To examine the solvent effect on the stability of a dianionic pentacoordinate intermediate, we carried out 3-21+G* level ab initio calculations[17] with respect to water-solvated pentacoordinate dianionic oxyphosphorane **3b**, the simplest possible (least stable) model compound of this type. Initially, we revised the ab initio gas-phase reaction profile for the reaction of $OH^- + H_2PO_4^- \rightarrow [H_3PO_5^{2-}] \rightarrow H_2PO_4^- + OH^-$ at 3-21+G* and 6-31+G* levels (see supplementary Table II for details). The reaction profiles are essentially the same as the previously calculated 3-21G* profile.[9] It is thus confirmed by higher-level calculations that *dianionic* **3b** does not exist along the reaction coordinate and the hydroxide attack and displacement proceed by a single-step mechanism via a pentacoordinate transition state. However, as we expected, the solvation of **3b** with water molecules allows *dianionic* **3b** to exist as the pentacoordinate intermediate on the potential surface.[18] The location of the solvated intermediate with six water molecules was carried out by two-step geometry optimizations. First, the solvated system was optimized under C_s symmetry constraints with respect to its equatorial plane. Next, starting from this C_s structure, full optimization was carried out without any symmetrical constraints at the 3-21+G* level. The frequency calculation ensured that the final structure had no imaginary frequencies. Thus, it is indeed a pentacoordinate intermediate (Figure 1).

The *dianionic* oxyphosphoranes **2a**,[7c] **3a**,[8] and **3c**,[6] possessing methoxyl groups, have some tendency to exist as intermediates

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(1) Cox, J. R., Jr.; Ramsey, B. *Chem. Rev.* **1964**, *64*, 317-352.

(2) Breslow, R.; Labelle, M. *J. Am. Chem. Soc.* **1986**, *108*, 2655-2659.

(3) Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473-4482.

(4) Taira, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1903-1909.

(5) Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872-5873.

(6) Dejaegere, A.; Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1991**, *113*, 4353-4355.

(7) (a) Taira, K.; Uebayasi, M.; Maeda, H.; Furukawa, K. *Protein Eng.* **1990**, *3*, 691-701. (b) Uchimaru, T.; Storer, J. W.; Tanabe, K.; Uebayasi, M.; Nishikawa, S.; Taira, K. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1523-1528. (c) Storer, J. W.; Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Nishikawa, S.; Taira, K. *J. Am. Chem. Soc.* **1991**, *113*, 5216-5219.

(8) Uchimaru, T.; Tanabe, K.; Nishikawa, S.; Taira, K. *J. Am. Chem. Soc.* **1991**, *113*, 4351-4353.

(9) (a) Taira, K.; Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Nishikawa, S. *Nucl. Acids Res.* **1991**, *19*, 2747-2753. (b) Yliniemela, A.; Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Taira, K. *Nucl. Acids Res. Symp. Ser.* **1992**, *27*, 57-58.

(10) (a) Uebayasi, M.; Uchimaru, T.; Taira, K. *Chem. Express* **1992**, *7*, 617-620. (b) Lim, C.; Tole, P. *J. Am. Chem. Soc.* **1992**, *114*, 7245-7252.

(11) (a) Uchimaru, T.; Tanabe, K.; Uebayasi, M.; Taira, K. *Chem. Express* **1992**, *7*, 501-504. (b) Uchimaru, T.; Uebayasi, M.; Tanabe, K.; Taira, K. *FASEB J.* **1993**, *7*, 137-142.

(12) Haake, P. C.; Westheimer, F. H. *J. Am. Chem. Soc.* **1961**, *83*, 1102-1109.

(13) Kluger, R.; Covitz, F.; Dennis, E.; Williams, L. D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1969**, *91*, 6066-6072.

(14) Brown, D. M.; Usher, D. A. *Proc. Chem. Soc.* **1963**, 309-310.

(15) Breslow, R.; Huang, D. L.; Anslyn, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1746-1750.

(16) (a) Gorenstein, D. G.; Chang, A.; Yang, J.-C. *Tetrahedron* **1987**, *43*, 469-578. (b) Taira, K.; Uchimaru, T.; Storer, J. W.; Yliniemela, A.; Uebayasi, M.; Tanabe, K. *J. Org. Chem.*, in press. (c) Since **2a** undergoes pseudorotation during alkaline hydrolysis, **2a** exists as an intermediate in basic media.[16a,b]

(17) In the present study, ab initio calculations were carried out at the RHF/3-21+G* and RHF/6-31+G* level of accuracy. Gaussian 90 [Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schlegel, H. B.; Raghavachari, K.; Robb, M. A.; 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*; Gaussian Inc.: Pittsburgh, PA, 1990] was used on IBM 6000/530 and on HP 700 workstations for the calculations.

(18) We tested the effects of one and two water molecules on the stability of the pentacoordinate intermediate **3b**. The presence of water molecules modified the potential surface to a remarkable extent, but no intermediate was found even after an extensive search. Thus, we decided to increase the number of water molecules immediately from two to six, which is the practical limit of our computational resources (optimization of **3b** with six water molecules took approximately 1 month of CPU-time on an IBM 6000/530 workstation). The minimum number of water molecules needed to support the existence of the pentacoordinate intermediate **3b** is unknown.

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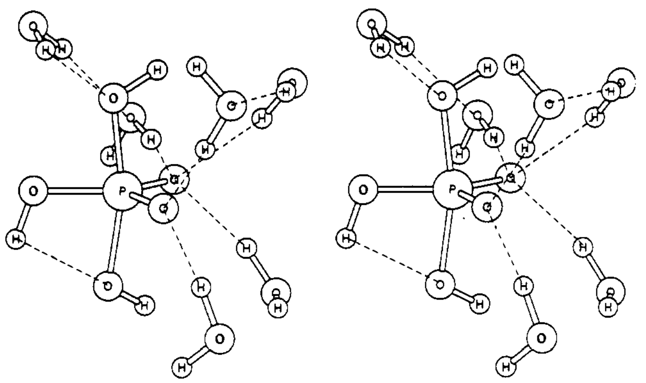

Figure 1. Stereoview of the fully optimized (3-21+G* level of accuracy) complex of dianionic oxyphosphorane **3b** with six water molecules. The hydrogen connected to O_3 (numbering is the same as in Scheme I) is located axially, with an $H-O_3-P_1-O_5$ dihedral angle of 2.7°. The internal geometries of the water molecules in the complex are slightly distorted (H–O–H angles 104.9–109.8°; O–H lengths, 0.963–1.012 Å) as compared with the geometry of the 3-21+G*-optimized water molecule (H–O–H = 111.0°; O–H = 0.964 Å). The dashed lines indicate O–H distances of less than 2.1 Å, suggesting an interaction between the hydrogen and the respective oxygen.

on the potential surface, whereas the smaller **2b**[5] and **3b**,[9] which have hydroxyl groups, exist only as transition states (calculated at the 3-21G* or 3-21+G* level). By contrast, *monoanionic* **2b** (3-21G*)[5] and **3b** (3-21+G* and 6-31+G*)[19] can both be located on the potential surface. The results of natural bond orbital (NBO) analysis for 3-21+G*-optimized transition structures of **3b** and **3a** suggest that methoxyl groups, as compared with hydroxyl groups, have a tendency to delocalize the negative charge.[20] The NBO analysis for water-solvated **3b** indicates that water solvation has a qualitatively similar effect to that of alkyl groups: it reduces the negative charge of **3b**, thus causing the *dianionic* compound to behave in a manner more characteristic of a stable *monoanionic* compound. The stability of the charged intermediate is, thus, greatly increased in the presence of water molecules by the delocalization of negative charge. The NBO charge of dianionic oxyphosphorane **3b**, when it is solvated with six water molecules, is reduced from −2.000 to −1.755. The water molecules accommodate part of the negative charge.

In conclusion, we have shown that even the least stable *dianionic* **3b** can exist, once it is sufficiently hydrated, as a stable pentacoordinate intermediate (Figure 2). Since naked *dianionic* **3b**, monohydrated **3b**, and dihydrated **3b** cannot exist as intermediates, the extent of hydration is, of course, important.[18] Nevertheless, the results presented here lead us safely to the conclusion that, in aqueous solution, when species are sufficiently hydrated, any pentacoordinate oxyphosphorane can exist as an intermediate. Thus, the lack of ^{18}O exchange and the lack of phosphoryl migration under basic conditions should be interpreted as a consequence of a high-energy barrier to pseudorotation,[9,21] and such results should not be taken as supportive evidence for the nonexistence of pentacoordinate intermediates. Solution-phase RNA hydrolysis and phosphate migration reactions can, thus, take place via a pentacoordinate oxyphosphorane intermediate not only under acidic but also under basic conditions.

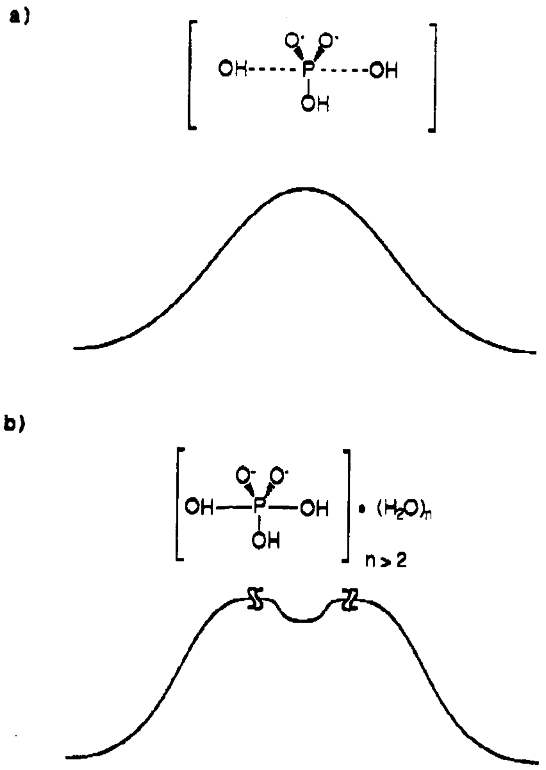

Figure 2. Schematic representations of reaction profiles for $OH^- + H_2PO_4^- \rightarrow [H_3PO_5^{2-}] \rightarrow H_2PO_4^- + OH^-$. (a) Ab initio calculations indicate that the pentacoordinate intermediate does not exist along the gas-phase reaction coordinate. Thus the reaction takes place in a single-step mechanism in the gas phase. The activation energy of the reaction is calculated to be 92 kcal/mol at the 3-21+G* level (the total energies of OH^-, $H_2PO_4^-$, and $[H_3PO_5^{2-}]$ are −74.995 74, −638.349 57, and −713.198 07 au, respectively). (b) However, the present work shows that solvation with water molecules allows dianionic oxyphosphorane species to exist as the pentacoordinate intermediate. Solution-phase phosphoryl-transfer reactions can thus take place via a pentacoordinate intermediate not only under acidic but also under basic conditions.

Supplementary Material Available: Table of NBO charges of **3a** and **3b**; table of geometries of **3b** (transition-state and water-solvated minimum) (2 pages). Ordering information is given on any current masthead page.

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[19] Yliniemela, A.; Uchimaru, T.; Taira, K., unpublished results.

[20] It must be emphasized that alkyl groups, as compared with hydrogens, are better able to stabilize both positively and negatively charged structures: Grimm, D. T.; Bartmess, J. E. *J. Am. Chem. Soc.* **1992**, *114*, 1227–1231.

[21] Wasada, H.; Hirao, K. *J. Am. Chem. Soc.* **1992**, *114*, 16–27. For the monoanionic oxyphosphorane that corresponds to **3b**, the barrier to pseudorotation in which the equatorial phosphoryl oxygen occupies the axial position is found to be twice as high as that to the pseudorotation, in which the equatorial phosphoryl oxygen remains in the equatorial position. The MP4//6-31G* barriers to pseudorotation are 11.64 and 6.19 kcal/mol, respectively. In the case of dianionic oxyphosphoranes, the negatively charged equatorial oxygen has to move from an equatorial to an axial position during the pseudorotation. Therefore, the pseudorotation barrier for dianionic oxyphosphorane can be expected to be higher than that for monoanionic or neutral species, and the possibility of cleavage of the P–O bond prior to pseudorotation is increased.