

Computational Study of the Influence of Solvent on $^{16}\text{O}/^{18}\text{O}$ Equilibrium Isotope Effects in Phosphate Deprotonation Reactions

Karin Kolmodin, Victor B. Luzhkov, and Johan Åqvist*

Contribution from the Department of Cell and Molecular Biology, Biomedical Center, Uppsala University, Box 596, SE-751 24 Uppsala, Sweden

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Abstract: Results from theoretical calculations of $^{16}\text{O}/^{18}\text{O}$ equilibrium isotope effects (EIEs) on deprotonation of phosphate and methyl phosphate monoanions as well as their deuterated counterparts are reported. The EIEs are calculated from the Bigeleisen equation using harmonic vibrational frequencies from several quantum mechanical methods (HF, DFT, MP2, and AM1). All methods correctly predict the qualitative trends in the EIEs related to the different isotope substitutions. However, the calculated gas-phase values are found to be systematically higher than those experimentally observed in aqueous solution. On the other hand, the addition of explicit solvent molecules (up to 24 waters) in the first solvation shells of the phosphate ion substantially improves the calculated EIE, which approaches the experimental value with increasing size of the water cluster. The large effects of surrounding water molecules on the phosphate deprotonation EIE can be explained by the strong solute–solvent interactions, which result in solvent coupled vibrational modes of the phosphate ions.

Introduction

Hydrolysis of phosphate esters is a fundamental reaction in biochemistry and a vast amount of research has been devoted to the study of phosphoryl transfer reactions, both in solution and catalyzed by enzymes.^{1–9} The use of ^{18}O isotope effects from the three nonbridging phosphate oxygens as a diagnostic tool for investigating phosphoryl transfer reaction mechanisms in solution was pioneered by Cleland and co-workers.^{10,11} They measured the secondary kinetic isotope effect (KIE) on hydrolysis of glucose-6-phosphate monoanion where the nonbridge phosphate oxygens were substituted by ^{18}O . Since a possible proton transfer involving one of the nonbridge oxygens may complicate the interpretation of monoanionic phosphate hydrolysis reactions, they also measured the equilibrium isotope effect (EIE) on deprotonation of phosphate and glucose-6-phosphate in order to examine such a contribution to the total measured kinetic isotope effect. For example, if one assumes a

dissociative hydrolysis mechanism preceded by preequilibrium proton transfer to the bridge oxygen, the measured KIE is likely to be dominated by the EIE on deprotonation of the phosphate group¹¹ (the same would be true for an associative mechanism with preequilibrium proton transfer from the nucleophile to the phosphate group, but with the EIE on protonation then presumably dominating the measured effect). At any rate, the correction of the experimentally obtained KIEs due to the EIE on deprotonation has apparently become a standard procedure for this type of experiment, where proton transfer to the bridge oxygen is usually assumed to be part of the mechanism in hydrolysis of the monoanion. The corresponding corrected ^{18}O -(V/K)_{nonbridge} KIE on enzyme catalyzed phosphate hydrolysis has become a widely used tool to characterize the nature of transition states involved in the different reaction mechanisms.^{12,13}

It is thus of considerable interest to investigate whether modern quantum chemical methods can reproduce the experimentally observed heavy atom isotope effects on phosphate reactions. If so, calculated KIEs may be used in future mechanistic interpretations of biologically important phosphate hydrolysis processes. Calculations of heavy atom isotope effects are clearly challenging from a theoretical point of view, and this topic is currently receiving increasing attention (see, e.g., refs 14–25 and further references therein). We recently

* Corresponding author. Phone: +46-18-471 4109. Fax: +46-18-536971. E-mail: aqvist@xray.bmc.uu.se.

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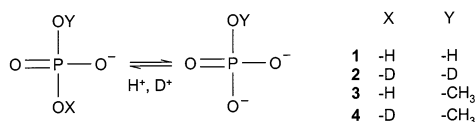


Figure 1. Chemical structures of different phosphate ions considered in this study. In compounds **1** and **2** the effects of substituting all four oxygens with ^{18}O are calculated, while for **3** and **4** the bridging oxygen atom bonded to Y is always ^{16}O .

reported²⁶ attempts to calculate heavy atom KIEs on monoanionic phosphate monoester hydrolysis in the gas phase and with a continuum solvent model.²⁷ It was found that neither a dissociative nor an associative reaction pathway could reproduce the experimentally observed normal (>1) KIE on phosphate monoanion hydrolysis in solution. The origin of such a discrepancy could possibly come from insufficiently accurate computational methods and/or inadequate models of the chemical system.

In this paper our objective is therefore to further investigate what models and methods are required in order to reproduce the experimental values for the simpler case of equilibrium isotope effects on phosphate deprotonation, which is highly relevant for the KIE on phosphate hydrolysis as mentioned above. The calculation of EIEs is presumably much less complex than calculations of KIEs because accurate transition structures, involving solvent molecules, do not need to be characterized. It is sufficient to only consider the stationary points of the reactant and product species respectively, i.e., the phosphate monoanion and dianion. The impact of different computational methods and solvent molecules on the isotope effect is then more straightforward to evaluate. To investigate these issues, we use here different quantum mechanical methods and levels of theory to calculate the effects of $^{18}\text{O}/^{16}\text{O}$ and H/D isotopic substitutions on phosphate deprotonation equilibria (Figure 1) in the gas phase and in an aqueous environment.

Methods

Quantum mechanical calculations of EIEs were performed using several levels of theory: ab initio Hartree–Fock (HF), the semiempirical AM1 method, second-order Møller–Plesset (MP2) perturbation theory, and density functional theory (DFT) at the level of the B3LYP functional. The HF calculations involved different basis sets of varying complexity (see Table 1), while the MP2 and DFT calculations were based on the 6-31+G(d) basis set. Calculations were performed both for the isolated ions and with account for solvent effects. The latter case was modeled by a limited number of explicit water molecules in a cluster. All structures were fully optimized in redundant coordinates with the predicted conformational minima being characterized by harmonic frequency calculations. The calculated frequencies were adjusted for the deficiencies of the theoretical approaches by the recommended scaling factors of 0.8929 for HF, 0.9613 for DFT, 0.9427

for MP2, and 0.9532 for the AM1 method.²⁸ The energy and frequency calculations were performed using the Gaussian-98 program.²⁹ The EIEs on deprotonation were calculated using the Bigeleisen equation derived directly from the partition function and the calculated harmonic vibrational frequencies ν at the stationary points:³⁰

$$\text{EIE} = \frac{K_{\text{eq}}^{\text{L}}}{K_{\text{eq}}^{\text{H}}} = \frac{\prod_{i=1}^{3N-6} u_i^{\text{H}}/u_i^{\text{L}} \prod_{i=1}^{3N-6} [(1 - e^{-u_i^{\text{L}}})/(1 - e^{-u_i^{\text{H}}})] e^{(\sum_i^{3N-6} (u_i^{\text{L}} - u_i^{\text{H}})/2)}}{\prod_{j=1}^{3M-6} u_j^{\text{H}}/u_j^{\text{L}} \prod_{j=1}^{3M-6} [(1 - e^{-u_j^{\text{L}}})/(1 - e^{-u_j^{\text{H}}})] e^{(\sum_j^{3M-6} (u_j^{\text{L}} - u_j^{\text{H}})/2)}} \quad (1)$$

Here u denotes $h\nu/kT$. Index i refers to the frequencies of the reactant (monoanion) and index j to the frequencies of the product (dianion). L denotes normal (light) and H heavy isotopes. N and M is the number of atoms in the reactant and product species, respectively. A number of studies (e.g. refs 14–25, 31) have demonstrated that ab initio vibrational frequencies can be successfully used in studies of isotope effects.

Results

The calculations show normal (>1) $^{16}\text{O}/^{18}\text{O}$ EIEs on deprotonation of phosphates **1** and **2** at all levels of theory, which is also found in the solution experiments (Table 1). The major contribution to the EIEs originates, as expected, from the zero-point vibrational energies (ZPVE). The semiempirical AM1 method gives significantly smaller EIEs compared to the methods of higher level. In the case of HF methods the value of the EIEs changes substantially only upon adding heavy atom diffuse functions to the split valence basis set with the heavy atom polarization functions, i.e., moving from the 6-31G(d) to the 6-31+G(d) basis set. Further addition of polarization functions to hydrogen atoms and the shift to triple split valence basis set do not result in particularly large changes in the calculated EIEs. Methods with partial account for electron correlation have a slight effect on the result. MP2 gives somewhat higher values than the high-level HF approaches, whereas B3LYP gives lower values. The values of the EIEs are smaller for methyl phosphates **3** and **4**, which have three substituted oxygen atoms, compared to the inorganic phosphates **1** and **2**, which for symmetry reasons have isotope substitutions at all four oxygens. The central phosphorus atom of the studied ions is bonded to four oxygen atoms, in which case the isotopic substitution of three or four oxygen atoms necessarily gives a mixture of primary and secondary isotope effects on deprotonation of one oxygen atom. Thus, the smaller EIE for **3** (and **4**)

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Table 1. Calculated and Experimental $^{16}\text{O}/^{18}\text{O}$ EIEs on Deprotonation of Phosphate and Methyl Phosphate Monoanions^a

ion	H_2PO_4	D_2PO_4	MeOPO_3H^b	MeOPO_3D^b
expt ¹⁰	1.0190	1.0248	1.0154	1.0220
calc ^c	Bigeleisen(ZPVE)	Bigeleisen(ZPVE)	Bigeleisen(ZPVE)	Bigeleisen(ZPVE)
	gas phase			
AM1	1.0289 (1.0245)	1.0366 (1.0375)	1.0232 (1.0202)	1.0308 (1.0321)
HF/6-31G(d)	1.0378 (1.0397)	1.0462 (1.0540)	1.0216 (1.0202)	1.0297 (1.0321)
HF/6-31+G(d)	1.0444 (1.0496)	1.0527 (1.0618)	1.0276 (1.0277)	1.0359 (1.0397)
HF/6-31+G(d,p)	1.0447 (1.0507)	1.0531 (1.0629)	1.0281 (1.0288)	1.0364 (1.0408)
HF/6-311+G(d,p)	1.0459 (1.0529)	1.0543 (1.0663)	1.0283 (1.0288)	1.0366 (1.0397)
HF/6-311+G(2d,p)	1.0459 (1.0518)	1.0542 (1.0652)	1.0282 (1.0288)	1.0365 (1.0397)
B3LYP/6-31+G(d)	1.0418 (1.0518)	1.0489 (1.0652)	1.0251 (1.0277)	1.0322 (1.0386)
MP2/6-31+G(d)	1.0466 (1.0535)	1.0545 (1.0660)	1.0287 (1.0292)	1.0365 (1.0407)

^a The experimental error bars are ± 0.001 .¹⁰ ^b Experimental data for glycerol-3-phosphate in solution. ^c EIE calculated using both the Bigeleisen equation and ZPVE contributions only. Frequencies scaled by 0.8929 for HF, 0.9427 for MP2, 0.9613 for DFT, and 0.9532 for AM1 calculations. $T = 300.15$ K.

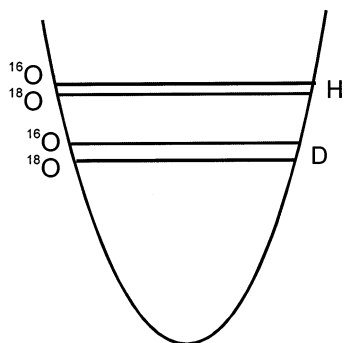


Figure 2. Illustration of the increased $^{16}\text{O}/^{18}\text{O}$ equilibrium isotope effect on phosphate deprotonation due to H/D substitution that affects the reactant primary vibration.

compared to **1** (and **2**) can be attributed simply to the fact that fewer atoms are substituted resulting in fewer stretching and bending frequency contributions to the overall isotope effect. The increased values of the $^{16}\text{O}/^{18}\text{O}$ EIE in deuterium substituted phosphates can mainly be explained, as a primary effect, by the increased reduced mass of the O–D oscillator relative to O–H, which results in a larger frequency split between the two oxygen isotopes in the reactant vibration (Figure 2). The EIEs calculated from ZPVE contributions only are also given in Table 1. In most cases these values are larger than the full EIEs calculated from eq 1. The differences reflect effects associated with taking into account excited vibrations as well as translational and rotational contributions in the Bigeleisen equation. Note, that the latter two are embedded in the first term of eq 1 through the Teller–Redlich product rule.³²

In general, the gas-phase calculations summarized in Table 1 agree qualitatively well with the experimentally observed trends in solution for the EIEs with respect to the different isotopic substitutions (**1**–**4**). However, all the calculated EIEs are systematically higher than the experimental ones, and the values do not show any tendency to improve upon increasing the level of theory. The use of unscaled vibrational frequencies in eq 1 makes this discrepancy even larger. These results clearly suggest that the gas-phase isotope effects are significantly different from those measured in aqueous solution and that the surrounding medium must be accounted for in the model.

Our next step is thus to bring solvent effects into the computational model through explicit phosphate–water complexes. The geometry of the phosphate ions is characterized by

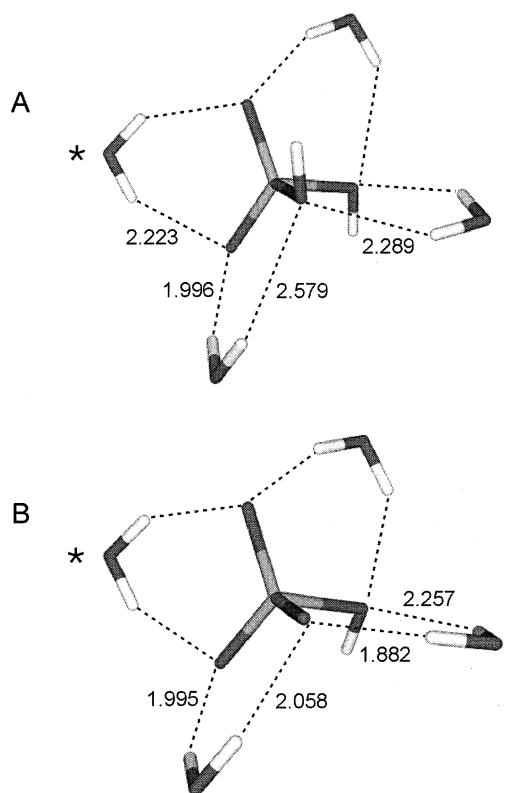
the four oxygen atoms forming a nonsymmetric tetrahedron around the central phosphorus atom with the P–O distances being 1.47–1.64 Å. Radial distribution functions generated from molecular dynamics simulations of the phosphate monoanion and dianion in water show that approximately 14 waters constitute the first solvation shell. The quantum mechanical calculations of such solute–solvent systems were performed for water clusters surrounding phosphate **1**. Geometry optimizations and frequency calculations with the higher level (correlated) methods were only done for smaller clusters, while the two least expensive methods (AM1 and HF/6-31G(d)) were used for the largest clusters.

Building of the solute–solvent clusters presents a rather complicated problem associated with the determination of multiple-minima potential energy surfaces for the intermolecular interactions. The procedure that we use here involves sequential addition of water molecules to the vacant positions around the solute's oxygen atoms, and optimizing the structures of such complexes. The global energy minimum of the complex with phosphate and one single water molecule corresponds to a symmetric configuration, where the water is bound by two H-bonds to the two oxygen atoms sharing the formal negative charge (Figure 3). In the symmetric four-water clusters, the water positions were selected such that all of them make double H-bonds with the solute (Figure 3). The addition of more waters to the solvation sites near the solute's oxygen atoms gradually covers the solvation surface of the phosphate ions. In most cases, the manual addition of waters causes effective reorientation and displacement of the water molecules from previously optimized positions. For example, in the clusters having more than nine waters, the solvent molecules do not stay in the double H-bonded position, which has the lowest energy for the one-water complexes, but prefer to form just a single H-bond with the solute and share other H-bonds with the neighboring waters. The building procedure ensures getting a monotonically larger H-bonded solvent network but, of course, the global minimum for each of the clusters can in general not be found. Nevertheless, such a straightforward approach provides a reasonable estimate of the solute–solvent and solvent–solvent H-bonding effects. In the largest clusters that we optimized using AM1, 24 water molecules covered the solvation surfaces of the monoanion and dianion of phosphate **1** without any apparent gaps (Figure 4). Structures of the dianion–water clusters were determined starting from the optimized structures of the corresponding monoanion clusters. In the dianion clusters the water molecules

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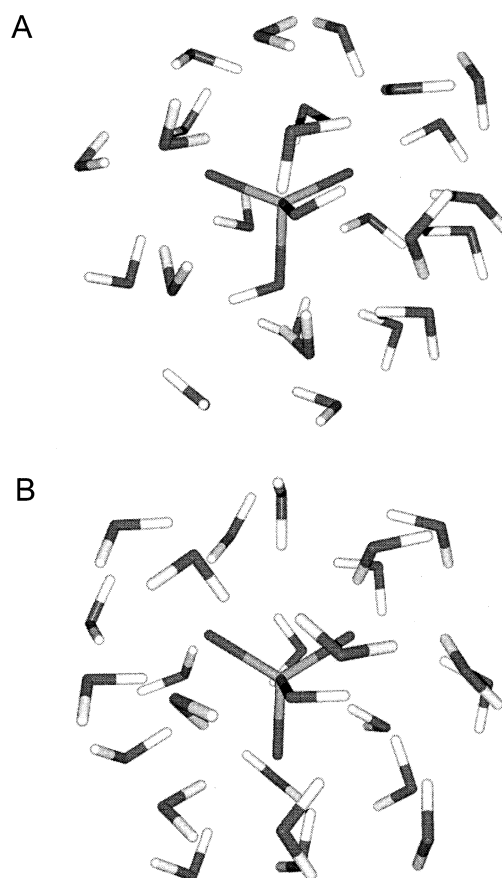
Table 2. Calculated Values of $^{16}\text{O}/^{18}\text{O}$ EIEs on Deprotonation of a Phosphate Monoanion in Clusters with Water Molecules

no. of waters	0	1	4	9	13	17	24
AM1	1.0289	1.0249	1.0183	1.0100	1.0110	1.0120	1.0167
no. of waters	0	1	4	5	9	14	17
HF/6-31 G(d)	1.0378	1.0340	1.0292	1.0285	1.0267	1.0228	1.0251
HF/6-31 +G(d)	1.0444	1.0363	1.0317	1.0309	1.0284		
B3LYP/ 6-31+G(d)	1.0418	1.0359	1.0295	1.0293			
MP2/6-3 1+G(d)	1.0466	1.0371					

**Figure 3.** 6-31G(d) optimized structures of (A) monoanion **1** and (B) dianion **1** with four waters. The asterisk (*) indicates the water position in the one-water cluster corresponding to the global minimum.

necessarily reorient themselves compared to the preceding positions in the solvation shell of monoanion. In particular, deprotonation of the OH group leads to reorientation of the water molecule that was H-bonded with the leaving proton. Obviously, the positions of the water molecules in the one-water and four-water complexes shown in Figure 3 are not subject to such reorientation.

Even the addition of one single water molecule to the phosphate hydration shell shifts the calculated value of the EIE to a comparable, or even larger, extent than the variations of theory level in the gas-phase calculations (Table 2). The corresponding drop in the calculated EIE is also largest for the cases that show the largest difference between the gas-phase value and experimental solution result. Increasing the size of the water cluster essentially gives a systematic decrease in the calculated EIE at all theory levels. The calculated values at the HF/6-31G(d) level gradually approach the experimental result (Table 2). The EIE obtained for the 14-water cluster equals 1.0228, which is already significantly closer to the experimental value of 1.0190 than to the gas-phase value of 1.0378. In the 17-water cluster with a completely different arrangement of solvent molecules around the solute the calculations show a

**Figure 4.** AM1 optimized structures of 24-water cluster with (A) monoanion **1** and (B) dianion **1**.

small increase in EIE (1.0251) compared to the 14-water cluster, but nevertheless the value is quite close to the experimental one. The difference in EIE between the 14- and 17-water clusters is probably related to a nonuniform solvation pattern of the phosphate in clusters of a limited size and gives an estimate of the error related to variations in the cluster configuration. The AM1 calculations altogether also perform fairly well. In this case, the calculated EIE successively drops from the gas-phase value of 1.0289 to 1.0100 for the 9-water cluster. Further increase of the number of water molecules gradually increases the EIE up to 1.0167 for the 24-water cluster (Table 2). In the latter complex, one can note that the innermost solvation shell(s) are essentially included (Figure 4). The calculated AM1 EIEs on deprotonation of either the first or the second OH groups are rather similar in this case (1.0164 and 1.0170, average 0.0167) which indicates that in the big clusters the OH groups of the phosphate start to become equivalent with respect to the predicted EIE. The average value of the EIE for the 24-water cluster, which is given in Table 2, is very close to the experimental value of 1.0190.¹⁰

Table 3. Influence of an Aqueous Environment on the Calculated Frequency of the Symmetric P–O Bond Stretch Vibrations of Unprotonated Oxygen Atoms in the Phosphate Monoanion and Dianion at the HF/6-31G(d) Level

method	freq ^a (cm ⁻¹)	
	monoanion	dianion
exp ^{33–36}	1040–1080	970–990
gas phase	1075	923
1-water cluster	1073	921
4-water cluster	1076	928
5-water cluster	1048	937
9-water cluster	1042	948
14-water cluster	1045	951
17-water cluster	1059	972

^a Frequencies scaled by 0.8929.

The profound effect of surrounding waters on the calculated EIEs is related to strong binding of water molecules to the phosphate ions. The estimated binding energy (at the MP2/6-31+G(d) theory level) of a single water molecule to monoanion **1** (in the lowest energy conformation) equals -17.9 kcal/mol, while the binding energy to the dianion is -33.2 kcal/mol. Such strong solute–solvent interactions necessarily cause strong coupling of the solute and solvent vibrational modes. For example, the primary O–H vibration of the phosphate monoanion is successively red-shifted from its scaled HF/6-31G(d) gas-phase value of 3685 cm⁻¹ as waters are added and stabilizes at clusters with nine or more water molecules. The predicted red shift of this vibration is about 300 cm⁻¹, and it reflects H-bonding of the phosphate protons to water oxygens. The primary O–H vibration of the phosphate monoanion is associated with an ¹⁶O/¹⁸O isotope effect of about 1.045 for the larger cluster sizes (1.059 in the gas phase). Hence, the overall EIE cannot simply be attributed to this stretching motion but apparently also depends to a large extent on the secondary vibrational modes in both the reactant and product.

As another example, one can examine the symmetric P–O bond stretch vibration in the mono- and dianion. Table 3 shows the calculated frequencies for these vibrations together with experimental results from IR and Raman spectroscopy in aqueous solution.^{33–36} It can be noted that there is a systematic shift of the dianion frequency toward higher wavenumbers with increasing number of surrounding water molecules. The 17-water cluster predicts an IR band in good agreement with the experimental value of about 980 cm⁻¹. The symmetric P–O stretching mode in the monoanion, on the other hand, is less affected by the solvent and somewhat red-shifted compared to the gas-phase value. The predicted frequency of about 1060 cm⁻¹ for the largest cluster is also in reasonable accord with the measured value. It can also be seen from Table 3 that there is a frequency shift associated particularly with adding the fifth water to the monoanion cluster, in which case the first phosphate proton becomes H-bonded to a water oxygen. Both of the above frequency shifts and, particularly, the “stiffening” of the product dianion thus contribute to a reduction of the ¹⁶O/¹⁸O EIE from its calculated gas-phase value. In the dianion, the average P–O

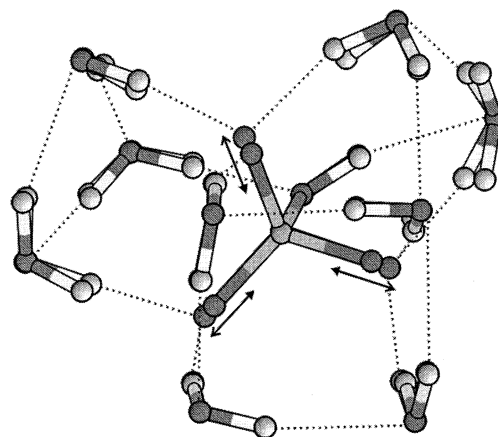


Figure 5. Illustration of the symmetric P–O bond stretch vibration and the coupled vibrational modes of the surrounding solvent molecules in the 9-water cluster.

bond order to the unprotonated oxygens is lower than in the monoanion and one can also expect more repulsion between the negative oxygen atoms. This leads to slightly elongated bonds and a red shift of the frequency (compared to the monoanion), as is indeed observed. The red shift is, however, reduced in the water clusters where both dielectric screening and charge transfer (evaluated as 0.2–0.3 electrons at the HF/6-31G(d) level for the 9-, 14- and 17-water clusters) to the solvent apparently cause a shortening and stiffening of the P–O bonds compared to the gas phase. In the cluster calculations the stretching mode is also coupled by H-bonding to rocking-like modes of the solvent molecules, which is illustrated for the 9-water cluster in Figure 5.

Conclusions

We have studied factors that influence the calculated value of ¹⁶O/¹⁸O EIEs on deprotonation of phosphate ions. The main finding is that the gas-phase calculations cannot reproduce the experimental results in solution, but yield systematically higher EIEs at the AM1, HF, DFT, and MP2 levels of theory employed in this study. This would necessarily lead to an inherent error in gas-phase calculations of kinetic isotope effects on phosphate monoanion hydrolysis, where the transition-state structures involve proton-transfer processes. We find in this work that the calculated EIEs quickly improve upon addition of explicit water molecules to the solvation shell. Our conclusions regarding the importance of microscopic solute–solvent interactions for predicting vibrational spectra and EIEs for these phosphate deprotonation reactions may also indicate that such a treatment can be useful in modeling the energetics of phosphate hydrolysis reactions. The ab initio and DFT values of the EIEs for the clusters approach the experimental value asymptotically from above, whereas the AM1 EIEs approach it from lower values. The substantial influence of the solvent on the EIEs is explained by strong binding of surrounding waters to the phosphate monoanions and dianions and a corresponding coupling of the solute–solvent vibrational modes. Limited attempts to account for the influence of the solvent on isotope effects at the microscopic level have been reported previously.^{37,38} Inclusion of a single^{37,38} or two³⁸ water molecules was found to improve

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the calculated H/D EIE on enolization of acetaldehyde³⁷ and the $^{16}\text{O}/^{18}\text{O}$ KIE on decarboxylation of 4-pyridylacetic acid zwitterion.³⁸ A comparison of microscopic and macroscopic approaches in the latter study³⁸ showed that the SM5.42 continuum solvent model³⁹ gave significantly better energetic results than the two-water cluster representation, while the obtained KIEs were of similar accuracy with the discrete and continuum representations. In the present case, the discrepancy

between the experimental solution data and theoretical gas-phase results for EIEs on deprotonation of phosphate ions is much larger and our calculations show that the effects of the aqueous environment are indeed very important for accurate modeling of these isotope effects.

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