

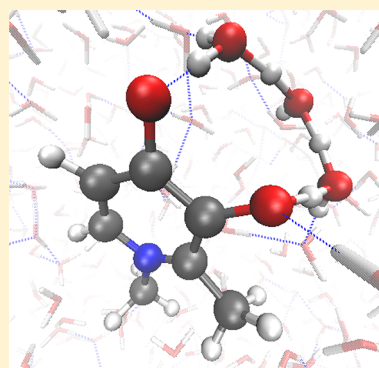
Prediction of Absolute Hydroxyl pK_a Values for 3-Hydroxypyridin-4-ones

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ABSTRACT: pK_a values have been calculated for a series of 3-hydroxypyridin-4-one (HPO) chelators in aqueous solution using coordination constrained ab initio molecular dynamics (AIMD) in combination with thermodynamic integration. This dynamics-based methodology in which the solvent is treated explicitly at the ab initio level has been compared with more commonly used simple, static, approaches. Comparison with experimental numbers has confirmed that the AIMD-based approach predicts the correct trend in the pK_a values and produces the lowest average error (~ 0.3 pK_a units). The corresponding pK_a predictions made via static quantum mechanical calculations overestimate the pK_a values by 0.3–7 pK_a units, with the extent of error dependent on the choice of thermodynamic cycle employed. The use of simple quantitative structure property relationship methods gives prediction errors of 0.3–1 pK_a units, with some values overestimated and some underestimated. Beyond merely calculating pK_a values, the AIMD simulations provide valuable additional insight into the atomistic details of the proton transfer mechanism and the solvation structure and dynamics at all stages of the reaction. For all HPOs studied, it is seen that proton transfer takes place along a chain of three H_2O molecules, although direct hydrogen bonds are seen to form transiently. Analysis of the solvation structure before and after the proton transfer event using radial pair distribution functions and integrated number densities suggests that the trends in the pK_a values correlate with the strength of the hydrogen bond and the average number of solvent molecules in the vicinity of the donor oxygen.



SECTION: Molecular Structure, Quantum Chemistry, and General Theory

INTRODUCTION

The rational design of high affinity, orally active iron chelators, as required, for example, for the effective treatment of iron-overloaded β -thalassemic patients¹ or those suffering from Friedreich's ataxia,² ideally requires that we have an accurate means to predict the pK_a values of the molecules' Fe^{3+} -coordinating functions. If a putative new chelator's pK_a value(s) can be predicted reliably, the expensive and time-consuming synthesis and testing of these compounds can be focused on those with a higher likelihood of providing clinical success—the predicted pK_a values, in turn, furnishing the means to estimate the compound's likely affinity for iron.³

The 3-hydroxypyridin-4-ones (HPOs, Figure 1) have been shown to be good therapeutic chelators, exhibiting the necessary affinity for Fe^{3+} along with good oral activity,⁴ and one such compound, Deferiprone (Figure 1), has emerged as a critically important therapeutic, able to remove accumulated excess iron from the heart^{5,6} and intracellular mitochondria.⁷ However, the search continues for other such synthetic chelators, with the aim to develop analogues that retain the excellent iron scavenging properties of Deferiprone, but lack its high metabolic susceptibility and undesirable lowering of white blood cell count.⁸

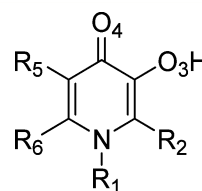


Figure 1. 3-Hydroxypyridin-4-one. Deferiprone (CP20), $R_1 = R_2 = CH_3$ and $R_5 = R_6 = H$ CP60, $R_1 = CH_3$ and $R_2 = R_5 = R_6 = H$ CP751, $R_1 = R_2 = R_5 = CH_3$ and $R_6 = H$ CP90, $R_1 = R_2 = R_5 = R_6 = H$.

To aid in this search, we sought here to explore the accuracy and reliability of the pK_a predictions that could be achieved for a series of HPOs using a number of existing, low-cost, quantitative structure property relationship (QSPR)^{9,10} and quantum mechanical (QM)¹¹ methods compared with full-blown ab initio molecular dynamics (AIMD) simulations.¹² The two former approaches respectively involve semiempirical estimates and single point calculations of the solute with solvent approximated as a dielectric continuum. The latter treat

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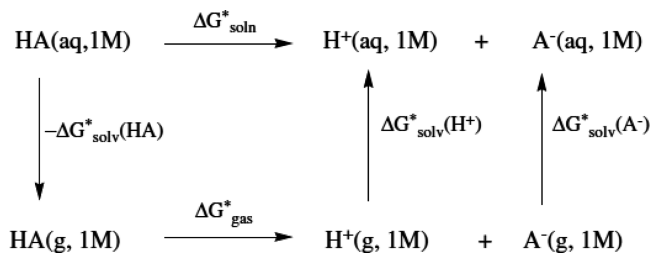
solute and solvent explicitly, on an equal footing, at the ab initio level and, in addition, take into account important entropic contributions by exploring the entire phase space.^{13–17} In fact, AIMD-based pK_a calculations require the simulation of the acid deprotonation process in aqueous solution. Beyond the mere pK_a prediction, AIMD-based approaches also provide valuable additional insights into the atomistic proton transfer mechanisms involved and the resolution of the solute's electronic structure at all stages of the reaction. The main challenge with AIMD-based pK_a evaluation is that the affordable time scale is typically many orders of magnitude shorter than that of acid dissociation. Hence, deprotonation is classified as a rare event in this context, and special sampling techniques^{18–23} must be employed. In the work reported here, we employ coordination constrained AIMD in combination with thermodynamic integration, a method that has been used successfully to calculate site-specific pK_a values of pentaoxyphosphoranes in aqueous solution.^{24,25} Thermodynamic integration has also been used successfully in AIMD-based pK_a calculations using other reaction coordinates.²⁶

COMPUTATIONAL METHODS

ACD/ pK_a DB 12.0 and Marvin 5.4.0.1 (QSPR) Methods. ACD/ pK_a DB 12.0⁹ and Marvin 5.4.0.1¹⁰ were used to predict the pK_a values of the compounds studied here. The ACD/ pK_a DB 12.0 method is derived from linear free energy relationships, using the empirical relations of Hammett and Taft.²⁷ The Marvin 5.4.0.1 method is derived from QSPRs, relating calculated structural descriptors to experimental pK_a values.²⁷

Static QM Calculations. Stable low energy structures for the HPOs were obtained through conformational searches performed at the semiempirical AM1 level using HyperChem Release 8.²⁸ The free energy of solution (ΔG^*_{soln}) for each compound was then computed using Gaussian 09²⁹—obtained using optimized geometries with no imaginary frequency, without symmetry constraints, with force constants calculated at every step and CPCM as a solvent model—and employing the thermodynamic cycles illustrated in Schemes 1 and 2. In the

Scheme 1. pK_a Prediction via Static QM Calculations Using the Simple Scheme¹¹



former (simple) scheme,¹¹ the experimental solvation free energy of the proton was taken as $\Delta G^*_{\text{solv}}(\text{H}^+) = -265.9$ kcal/mol.¹¹ In the computations performed using Scheme 2 (the proton exchange scheme),³⁰ the reference acid (HRef) was chosen as CP90.³¹ pK_a values were converted as

$$pK_a = \Delta G^*_{\text{soln}} / [RT \ln(10)]$$

where R is the gas constant (1.99 cal Kelvin⁻¹ mol⁻¹), T is the absolute temperature, and K is the molar equilibrium constant.

Coordination Constrained Car–Parrinello Molecular Dynamics Simulations. Constrained Car–Parrinello molec-

ular dynamics (CP-MD) simulations with the CPMD software³² were carried out for the three different solutes (CP20, CP60, and CP751) surrounded by 61 H₂O molecules in a periodically repeating cubic unit cell of length 12.4 Å. The Kohn–Sham equations were solved using the PBE³³ exchange–correlation functional in a plane wave basis set truncated at 25 Ry in conjunction with ultrasoft pseudopotentials.³⁴ The coupled nuclear and fictitious electronic equations of motion were solved using a time step of 6 au, setting the fictitious orbital mass to 800 au, and replacing all hydrogens with deuterium. A Nosé–Hoover chain thermostat^{35,36} was applied to maintain the nuclear temperature at 350 K. It has been shown that a slightly raised temperature is necessary in CP-MD simulations with this type of exchange–correlation functional to reproduce structural and dynamical experimental data for room-temperature liquid water.^{37–39}

The number of hydrogens coordinated to the hydroxyl oxygen of the solute was calculated using the formula

$$n = \sum_i \frac{1}{\exp[\kappa(r_i - r_c)] + 1} \quad (1)$$

with a cutoff radius of $r_c = 2.46$ au and $\kappa = 5.29$ au⁻¹, with r_i being the distance between the hydroxyl oxygen and the i th proton. For each fixed proton coordination number n , a trajectory of at least 1.5 ps was calculated, and the time-averaged constraint force $\langle f \rangle$ was determined. Each simulation was run until the running average of the constraint force reached a relative standard deviation of less than 2% over the last 700 fs.

The free energy difference between the initial equilibrium state with the hydroxyl group intact, described by the coordination number n_0 , and the final state, n_f at the top of the reaction barrier was obtained by thermodynamic integration:

$$\Delta A = A(n_f) - A(n_0) = \int_{n_0}^{n_f} \langle f'(n) \rangle dn \quad (2)$$

where $\langle f'(n) \rangle$ is the potential of mean force of the metrically corrected time-averaged constraint force⁴⁰ $\langle f \rangle$. Following the procedure developed by Chandler,^{24,41} we convert the free energy difference to an acidity constant using the relation

$$K_a = \frac{(1 - P)^2}{PC_0V} \quad (3)$$

where

$$P = \frac{\int_0^{R_c} 4\pi r^2 \exp(-\Delta A/kT) dr}{\int_0^{R_f} 4\pi r^2 \exp(-\Delta A/kT) dr} \quad (4)$$

is the probability of finding a proton within the radius R_c , C_0 is the standard concentration (1 mol/dm³), and V is the volume of the simulation cell. The value for the distance at which the hydroxyl OH dissociates was estimated from the position of the first minimum of the OH partial radial distribution function to be $R_c = 1.3$ Å, while R_f was taken to be the OH distance at the final coordination number n_f .

RESULTS AND DISCUSSION

The top panel of Figure 2 shows the potential of mean force (as obtained from the CP-MD simulations) as a function of the hydrogen coordination number of the HPO hydroxyl oxygen.

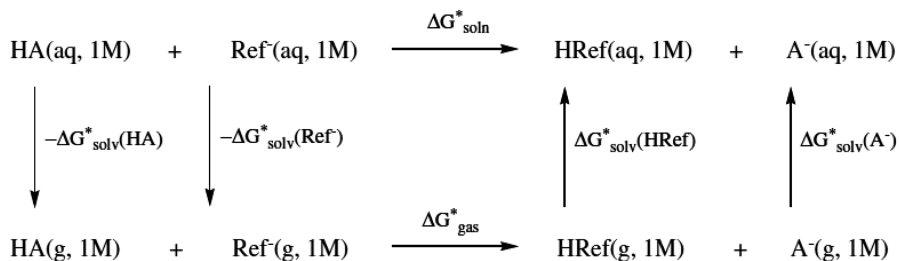
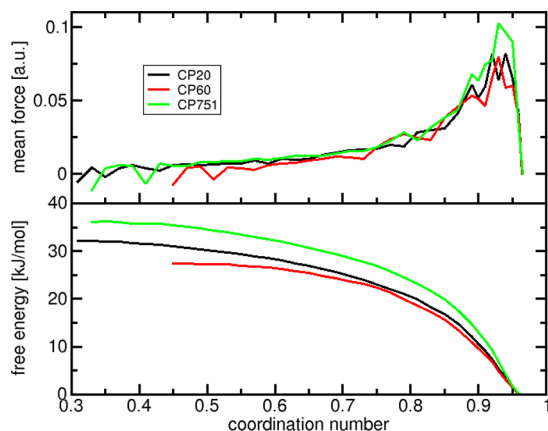
Scheme 2. pK_a Prediction via Static QM Calculations Using the Proton Exchange Scheme^{30a}^aHRef: a reference acid, CP90.

Figure 2. Mean force (top panel) and corresponding free energy (bottom panel) versus proton coordination number for the three different compounds: CP20 (black line), CP60 (red line), CP751 (green line).

The simulations were started at thermal equilibrium with the OH group fully intact, corresponding, according to our definition, to a coordination number of about 0.96. As one would expect, the mean force is practically zero at this point. Upon decreasing the coordination number, the OH bond is stretched, resulting in a sharp rise in the constraint force until it reaches a maximum at $n = 0.93$. Decreasing n further, the mean force first goes to zero as the system approaches the transition state for proton transfer before becoming negative as the system enters the product potential well. Snapshots illustrating the various stages of the proton transfer reaction at different values of the coordination constraint are shown in Figure 3.

Since only the region up to the transition state is relevant for the free energy barrier and thus for the pK_a value, it is sufficient for our quantitative analysis to take into account coordination numbers down to 0.3 only. Comparing the force curves for the three different compounds, CP751 shows the highest peak, followed by CP20 and, slightly lower, CP60. This order is reflected in the corresponding free energy profiles presented in Figure 2 (bottom panel), which clearly shows that CP751 has the largest free energy barrier ($\Delta A = 36.3$ kJ/mol), CP60 has the lowest ($\Delta A = 27.5$ kJ/mol), and CP20 lies in between with $\Delta A = 32.1$ kJ/mol. According to the procedure outlined above, these free energies have been converted to the pK_a values 9.00 (CP60), 9.36 (CP20), and 10.73 (CP751). In Table 1, our pK_a predictions from this work for the three test compounds, CP20, CP60 and CP751, are compared to those obtained via other theoretical methods and also experimental measurements.^{31,42} It can be seen that while the ACD/ pK_a DB 12.0 method gives good predictions for CP20 and CP751, it is significantly in

error for CP60, with the measured and predicted pK_a values differing by almost 1 log unit. The Marvin 5.4.0.1 method yields very poor predictions and overestimates the pK_a values for all three test compounds, again by about 1 log unit. The static QM calculations using the simple scheme gave pK_a values that followed the correct trend but with the absolute predicted values, in all three cases, overestimated by more than 6 log units. The static QM calculations using the proton exchange scheme corrected the absolute predicted values derived from the simple scheme, reducing the average absolute error from 6.25 to 0.99, but still, therefore, yielding pK_a values of insufficient accuracy. Compared to the other prediction methods, the CPMD method yields the best predictions with respect to trend and average absolute error from the measured values for all three compounds.

In the following, we attempt to correlate the differences in the acidity of the three species to their structural and dynamical properties. To begin with, we have analyzed the distances of the transferring hydrogen to the donor oxygen (O_3) and the acceptor oxygen (O_A) as a function of the O_3 coordination number (see Figure 4). Near thermal equilibrium, i.e., $n \approx 0.97$, the hydroxyl OH bond length is seen to be at its equilibrium value of about 0.99 Å for all three species, whereas the $H \cdots O_A$ hydrogen bond length exhibits considerable variation. By far, the shortest average hydrogen bond of around 1.7 Å occurs for CP60, in line with the fact that the simulations predict the highest acidity for this compound. The weakest hydrogen bond with a length of about 2.1 Å on average is displayed by CP751, which again seems to make sense as this compound has the highest pK_a value. Between those extremes lies CP20, as far as both the hydrogen bond length (2.0 Å) and the pK_a are concerned. These differences in hydrogen bond length among the three HPOs may perhaps be explained by the differences in the water hydrogen bonding networks surrounding their hydroxyl O_3 atoms, which arise because of their differing patterns of methyl substitution, which lead to differing numbers of water molecules within a radius of about 3 Å from O_3 (see Figures 5, bottom panel, and 6a). Interestingly, for all three HPOs, the crossover point at which the transferring H is midway between O_3 and O_A coincides at $n = 0.72$, where the OH distance is around 1.25 Å. Beyond the transition state, the curves for the three HPOs diverge slightly, indicating that CP60 forms the tightest HO_A bond followed by CP20 and CP751.

In the search for correlation between the acidity of a certain species and its solvation properties, we have also calculated partial radial pair distribution functions (RDFs) between the donor oxygen O_3 and water oxygens. In Figure 5, the O_3 –O RDFs for the three species are shown at the coordination number of 0.97. The first RDF peak of CP60 is much higher and the separation between the first and second peaks is much

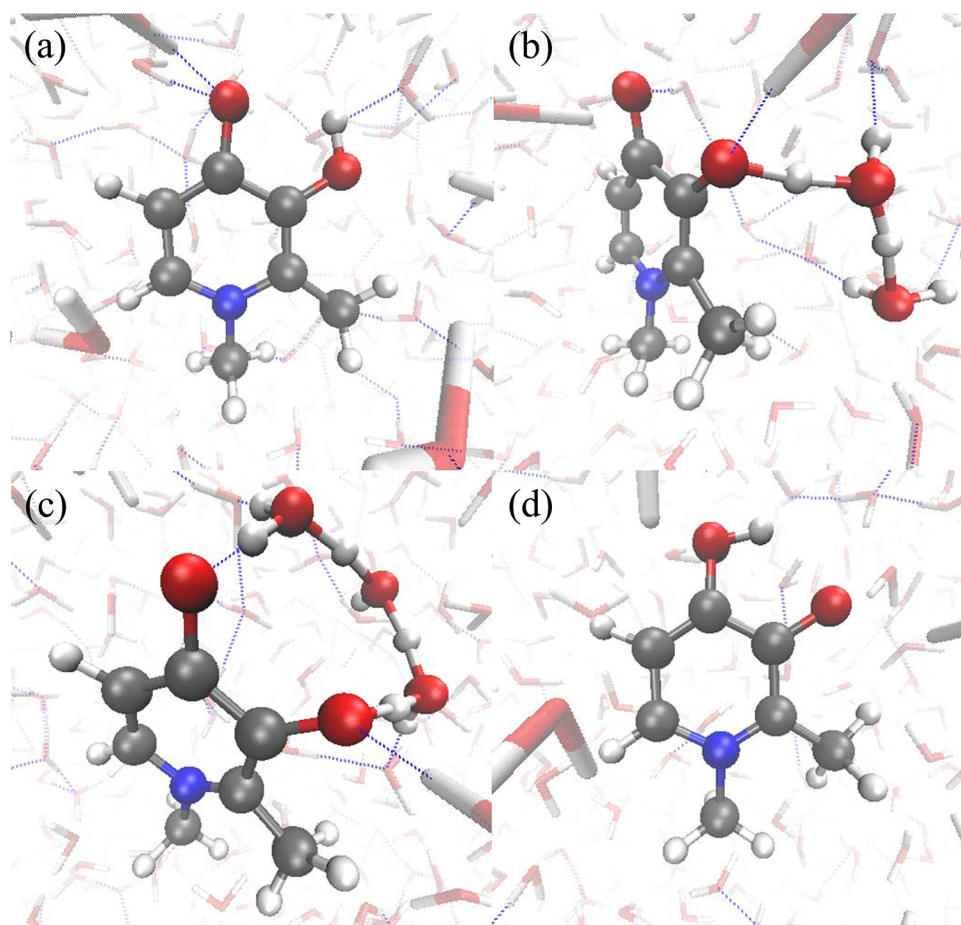


Figure 3. Snapshots from the simulation of CP20 in water illustrating the deprotonation process induced by the coordination constraint. (a) $n = 0.97$: the proton is covalently bonded to the O_3 atom with a hydrogen-bond to a nearby H_2O molecule; (b) $n = 0.71$: the proton is shared between the O_3 atom and the nearby H_2O molecule; (c) $n = 0.31$: a chain of 3 H_2O molecules forms a bridge mediating a PT chain reaction between the O_3 and O_4 atoms; (d) $n = 0.01$: the O_3 atom is completely deprotonated, while the O_4 atom is now protonated.

Table 1. pK_a Prediction Results Using Different Methods

	CP60	CP20	CP751	average absolute error
measured value	8.79 ³¹	9.76 ³¹	10.31 ⁴²	
CPMD	9.00	9.36	10.73	0.34
ACD/ pK_a DB 12.0	9.59	9.90	10.03	0.41
Marvin 5.4.0.1	10.79	10.72	10.71	1.12
simple scheme using B3LYP/6-31+G(d)/CPCM	14.32	15.90	17.36	6.25
proton exchange scheme using B3LYP/6-31+G(d)/CPCM	9.07	10.65	12.11	0.99

more pronounced than for the two other species, indicating a much stronger solute–solvent interaction compatible with its high acidity. Although the first peak for CP751 is higher than that for CP20, the number of water molecules within a radius of about 4 Å from its O_3 is significantly lower because of the 5-methyl substituent (see Figures 5, bottom panel, and 6a).

In addition to providing atomistic level insight into the solvation and proton transfer properties of the different initial species, the simulations also offer the possibility to characterize the final product state after proton transfer. Using the methodology applied in this work, it would be possible to determine the pK_a values of the carbonyl oxygen O_4 , which at the end of the simulation (i.e., for $n \rightarrow 0$) is fully protonated.

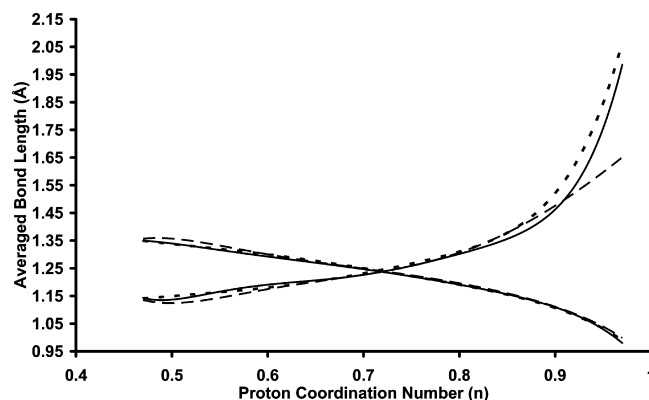


Figure 4. Mean OH distances between the transferring H and the donor (O_3) and acceptor (O_A) oxygen as a function of O_3 proton coordination number. CP20 (solid line), CP60 (dashed line), CP751 (short-dashed line).

However, a quantitative analysis of this sort is beyond the scope of this article. Instead, we simply attempt to rationalize the experimentally measured pK_a values of 3.66,³¹ 3.38,³¹ and 3.28³¹ for CP20, CP60, and CP751, respectively, on the grounds of their solvation structure. The O_4 –O RDFs in Figure 7 (top panel) show that CP751 forms stronger hydrogen bonds at O_4 , the first peak appearing at a shorter distance compared to

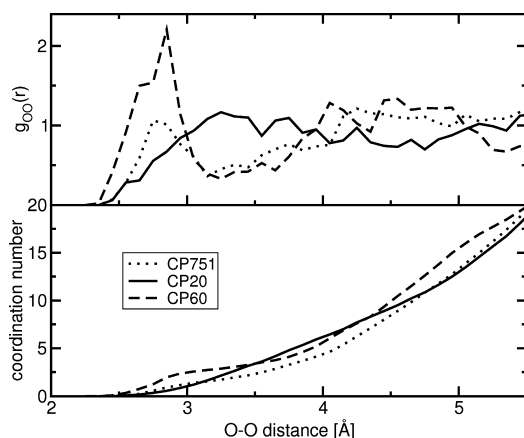


Figure 5. O_3 -O (H_2O) pair radial distribution function for the solvated HPOs with a mean proton coordination number of 0.97: CP20 (solid line), CP60 (dashed line), CP751 (short-dashed line).

the other two species, which may explain the slightly higher acidity of CP751. It is also apparent that the RDF for CP20 exhibits a much more pronounced dip between the first and second peaks which means that the first and second solvation shells are well separated. As a consequence, the integrated number density around O_4 is significantly lower for CP20 than for the other two species up to a distance of about 4.5 Å (see Figures 7, bottom panel, and 6b), and this may correlate with the decreased acidity of CP20. Comparison of the bottom panels of Figures 5 and 6 further shows that the differences in the solvent density are much smaller at O_4 than at O_3 , explaining the smaller differences in the pK_a values of the O_4H group.

CONCLUSIONS

We have calculated the pK_a values for a series of 3-hydroxypyridin-4-one chelators in aqueous solution using coordination constrained AIMD in combination with thermodynamic integration. This dynamics-based methodology in which the solvent is treated explicitly at the ab initio level has been compared with more commonly used simple, static, approaches. Comparison with experimental numbers has confirmed that the AIMD-based approach predicts the correct

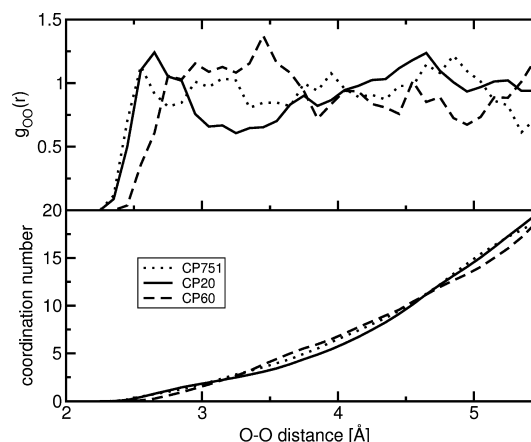


Figure 7. O_4 -O (H_2O) RDF for the solvated HPOs with a mean proton coordination number of 0.01, i.e., near the proton transfer product: CP20 (solid line), CP60 (dashed line), CP751 (short-dashed line).

trend in the pK_a values and produces the lowest average error. The corresponding pK_a predictions made via static QM calculations overestimate the pK_a values by 0.3–7 pK_a units, with the extent of error dependent on the choice of thermodynamic cycles employed. The use of simple QSPR methods gives prediction errors of 0.3–1 pK_a units, with some values overestimated and some underestimated.

Beyond merely calculating pK_a values, the AIMD simulations provide valuable additional insight into the atomistic details of the proton transfer mechanism and the solvation structure and dynamics at all stages of the reaction. We have seen that, for all three species, proton transfer takes place along a chain of three H_2O molecules, although direct hydrogen bonds are seen to form transiently. Analysis of the solvation structure before and after the proton transfer event using RDFs and integrated number densities suggests that the trends in the pK_a values correlate with the strength of the hydrogen bond and the average number of solvent molecules in the vicinity of the donor oxygen.

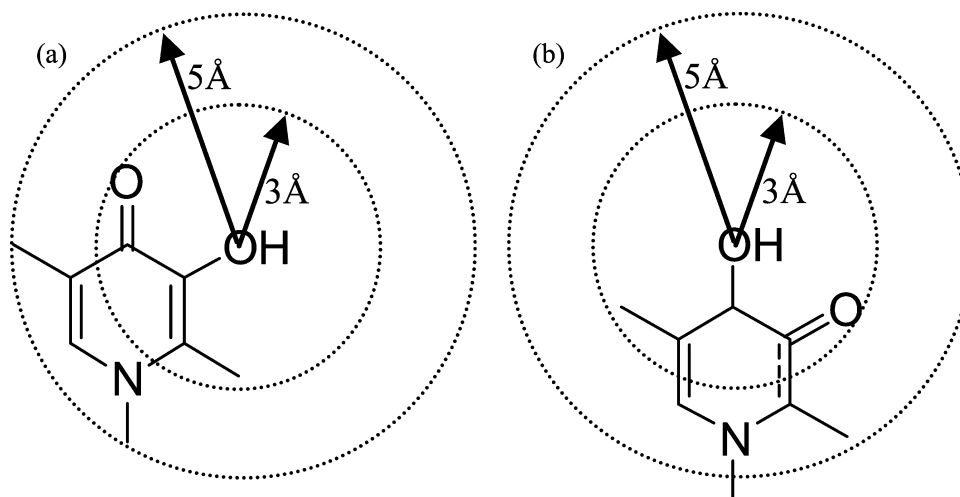


Figure 6. Schematic diagram illustrating the 3 Å and 5 Å HPO coordination radii with origin (a) at O_3 ; (b) at O_4 .

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Notes

The authors declare no competing financial interest.

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