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Estimating successive pK_a values of polyprotic acids from *ab initio* molecular dynamics using metadynamics: the dissociation of phthalic acid and its isomers

*Anil Kumar Tummanapelli and Sukumaran Vasudevan**

Department of Inorganic and Physical Chemistry

Indian Institute of Science, Bangalore 560012,

INDIA

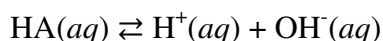
* Author to whom correspondence may be addressed. E-mail: svipc@ipc.iisc.ernet.in. Tel: +91-80-2293-2661. Fax: +91-80-2360-1552/0683;

ABSTRACT

Estimation of the dissociation constant, or pK_a , of weak acids continues to be a central goal in theoretical chemistry. Here we show that *ab initio* Car-Parrinello molecular dynamics simulations in conjunction with metadynamics calculations of the free energy profile of the dissociation reaction can provide reasonable estimates of the successive pK_a values of polyprotic acids. We use the distance-dependent coordination number of the protons bound to the hydroxyl oxygen of the carboxylic group as the collective variable to explore the free energy profile of the dissociation process. Water molecules, sufficient to complete three hydration shells surrounding the acid molecule, were included explicitly in the computation procedure. Two distinct minima corresponding to the dissociated and un-dissociated states of the acid are observed and the difference in their free energy values provides the estimate for pK_a , the acid dissociation constant. We show that the method predicts the pK_a value of benzoic acid in good agreement with experiment and then show using phthalic acid (benzene dicarboxylic acid) as a test system that both the first and second pK_a values as well, as the subtle difference in their values for different isomers can be predicted in reasonable agreement with experimental data.

INTRODUCTION

Knowledge of the acid dissociation constants of the ionizable protons in molecules in aqueous media is of fundamental importance in many areas of chemistry and biochemistry. The dissociation of an acid, HA, in aqueous solution may be represented in the form



and the $\text{p}K_a$ value defined as

$$\text{p}K_a = -\log(K_a) = \Delta G_a / 2.303 RT$$

where K_a is the equilibrium dissociation constant. The $\text{p}K_a$ value of a molecule determines the relative concentration of its protonated and deprotonated forms at a specific pH and is therefore an important descriptor of its chemical reactivity. Consequently, considerable efforts have been devoted to the experimental determination of $\text{p}K_a$ values. Although in many cases accurate experimental measurements are easily made, there are situations where accurate measurements are difficult. It is, therefore, not surprising that the capability to provide accurate estimates of the $\text{p}K_a$ value has been a central goal in theoretical chemistry and there has been a large effort in developing methodologies for predicting $\text{p}K_a$ values in a variety of chemical systems by differing quantum chemical techniques.¹⁻³ The prediction accuracy within 0.5 $\text{p}K_a$ units of experiment is the desirable level of accuracy. This is a non-trivial exercise for an error of 1 kcal/mole in estimates of the free energy value would result in an error of 0.74 $\text{p}K_a$ units.⁴

The majority of $\text{p}K_a$ calculations involve constructing the appropriate thermo-chemical acid dissociation cycle followed by evaluation of the free-energy change at each step of the cycle.¹ The dissociation free energy is expressed as the sum of the dissociation free energy of the molecule in the gas phase and the solvation free energy difference of the neutral acid and the dissociated products. In most calculations the gas phase acid dissociation is computed at the

quantum mechanical (QM) level while the solvent modeled as a dielectric continuum.⁵ The drawback of these procedures, and a major source of error, is that short-range intermolecular interactions with water molecules, such as hydrogen bonds and ion-dipole interactions as well as solvent dynamics, are not explicitly accounted for although they are particularly important, as these calculations involve ionic species. By far the most challenging aspect in the theoretical prediction of pK_a values is to account for the solvent in a physically meaningful way. A number of different strategies have been developed to improve the accuracy of the calculations by explicit inclusion of solvent water molecules in the acid dissociation process.⁶⁻⁸ For strongly coupled solute-solvent systems explicit inclusion of water molecules in the quantum mechanical part of the calculation can greatly improve the accuracy in computed pK_a values, especially for certain continuum models, such as the SM6 solvation model.³ A number of hybrid approaches have been developed to capture the first and second solvation directed effects in the context of continuum models. These have been summarized in recent reviews.^{3,9-12} An alternate approach to the continuum models is to model the solvent molecules using molecular mechanics (MM) using an appropriate force fields while describing the bond-breaking by QM procedures.¹³⁻¹⁷

A critical issue in any dissociation event is how the solvating water molecules arrange themselves both spatially and dynamically around the ionizable and ionized species. This, in principle, is best described by *ab initio* molecular dynamics wherein all solvent molecules in the ensemble are treated explicitly.¹⁸ In these simulations the electronic structure calculations are usually based on density functional methods and although these are more restricted as compared to the methods used in the hybrid continuum models have the advantage that solvation effects are handled with greater rigor. Consequently, a number of investigations of acid dissociation using *ab initio* molecular dynamics including Car-Parrinello molecular dynamic

have been reported.¹⁹⁻²² The systems investigated include HCOOH, CH₃COOH and H₂CO₃.¹⁹⁻²² It has also been reported that free energies associated with the dissociation of HCl, HCOOH, H₂S and *ortho* and *para* salicylic acids can be estimated from *ab initio* MD simulations using the distribution of the vertical energy gap for the insertion or deletion of a proton.²³ The dissociation of histidine in aqueous media has been explored using constrained *ab initio* Car-Parrinello molecular dynamics (CPMD).²² Free energies were obtained by integrating the potentials of mean force along a chosen coordinate for the deprotonation of histidine. Although the chosen reaction coordinate limited their analysis to stopping just beyond the transition state, the correct relative pK_a value was obtained by subtracting the equivalent profile for a reference reaction, the auto-dissociation of water.

One of the factors that has limited the use of *ab initio* MD methods is that dissociation of weak acids is an infrequent event and consequently unreasonably long simulation times are required before one is observed, making such studies impractical. One of the successful techniques to handle rare and infrequent events in MD simulations is the metadynamics formalism.²⁴⁻²⁶ The formalism allows the system to escape the free-energy minima, by biasing the dynamics with a history dependent potential (or force) that acts on select degrees of freedom, referred to as collective variables. The bias potentials, modeled by repulsive inverted Gaussians that are dropped during propagation, drive the system out of any free energy minima and allow it to explore the configurational space. The system is prevented from revisiting regions of configuration space where it has been in the past making the sampling relatively quick and efficient. It is therefore ideally suited for studying dissociation of weak acids. CPMD in combination with metadynamics has been successfully used to describe the dissociation of acetic acid in water.¹⁹ The dissociation reaction occurred along a well-characterized pathway and free

energy minima corresponding to the associated and dissociated states of acetic acid were observed along with a shallow minimum in the free energy surface corresponding to a contact ion pair. It was subsequently shown that the dissociation constant can be obtained, without the need of constructing thermo-chemical cycles, as the difference in the values of the free energy minima corresponding to the associated and dissociated states in the metadynamics computed free energy profile.²⁷ It had also been demonstrated that the formalism could correctly account for the influence of the inductive effect as well as hydrogen bonding on pK_a values of weak organic acids in good agreement with experiment.²⁷

Here we show that ab *initio* Car-Parrinello Molecular Dynamics (CPMD)²⁸ in conjunction with metadynamics computation²⁴⁻²⁶ of the free-energy landscape can provide accurate estimates of the successive pK_a value of polyprotic acids. This is of practical relevance for in many situations where successive dissociation constants are not well separated making accurate measurements of individual pK values difficult. We show that the dissociation constant of benzoic acid in water obtained from our calculations is comparable to experiment and then show using phthalic acid (benzene dicarboxylic acid) as a test system that both the first and second pK_a values as well, as the subtle difference in their values for different isomers can be predicted in reasonable agreement with experimental data.

SIMULATION METHODOLOGY

The CPMD v3.11.2²⁹ program was used to perform the ab *initio* molecular dynamics simulation. Simulations were performed on an IBM Blue Gene cluster using 256 (128 × 2) processors. A single acid molecule, either benzoic or phthalic acid, solvated by water molecules, was placed in a cubic box with periodic boundary conditions; the dimensions reproduce the experimental

density of water. All water molecules are considered explicitly in the CPMD simulations. The number of water molecules solvating the acid molecule was chosen such that at least three hydration shells were complete. The dimension of the simulation box was selected so as to ensure that after subtracting the van der Waals volume of the organic acid the space available for the water molecules would correspond to the experimental density of unity. Maintaining water density as close to unity is an important consideration for obtaining accurate pK_a values.²⁷ The number of water molecules and the dimension of the simulation cell are different for each organic acid and are specified in the Supplementary Information, Table S1.

The gradient corrected exchange correlational DFT-HCTH/120³⁰ functional was used in the present study because it provides significantly improved energies as compared to DFT-BLYP (Becke exchange and Lee-Yang-Parr correlation) for water-ion systems.³¹ Dispersive interactions were accounted for by the empirical dispersion correction of Grimme.³² Core electrons were treated using the normconserving atomic pseudopotentials of Troullier-Martins,³³ while valence electrons were represented in a plane wave basis set truncated at an extended energy of 70 Ry. The fictitious electron mass parameter was equal to 800 a.u.. The time step applied during the simulations was set to 6 a.u. (0.146 fs). All simulations were performed at a temperature of 300 K. The Nose-Hoover thermostat was used to control the temperature of the NVT ensemble. The initial part of the simulations was taken as the equilibration time (40 ps) and was not considered during the data analysis.

The metadynamics formalism in its extended Lagrangian version was employed to explore the free-energy profile of the dissociation process as defined by the collective variable n_{OH} , the O-H bond-distance dependent number of hydrogens coordinating the hydroxyl oxygen of the carboxylic group.

$$n_{\text{OH}} = \sum_{i=1}^{N_H} \frac{1 - \left(\frac{r_{\text{OH}_i}}{r_c}\right)^6}{1 - \left(\frac{r_{\text{OH}_i}}{r_c}\right)^{12}} \quad (1)$$

The collective variable n_{OH} has a value close to unity in the un-dissociated state and is zero on dissociation. The instantaneous O-H bond distance is r_{OH} and r_c ($= 1.6 \text{ \AA}$) is the critical, or “cut-off”, distance beyond which the O-H bond breaks. The exponents controls the long-range behaviour and stiffness and ensures a smooth decay of the coordination number.²⁵ The free energy profile was sampled by placing history-dependent Gaussian bias potentials along the dynamical time evolution path. The height and width of the Gaussian bias potentials were 0.0005 and 0.1 a.u., respectively, with the deposition time step 0.04 ps. The sampling was continued till the entire free-energy profile was explored and the motion in the collective variable space was diffusive. For simulations of the dissociation of phthalic acid n_{OH} is defined for a designated carboxylic group. For the second dissociation of phthalic acid the simulations were repeated but now with the phthalate ion and the collective variable the n_{OH} of the un-dissociated carboxylic group. For the first dissociation of phthalic acid the collective variable is designated as CV1 and for the second as CV2. The presence of the hydronium ion formed following the first dissociation ensures charge neutrality of the simulation cell for the computation of the second dissociation constant.

RESULTS AND DISCUSSIONS

The free-energy profile for the dissociation of benzoic acid is shown in Figure 1a. During the metadynamics simulations dissociation is observed within 6 ps. Two well-defined minima are observed; the minimum on the left corresponds to the un-dissociated neutral acid and that on the

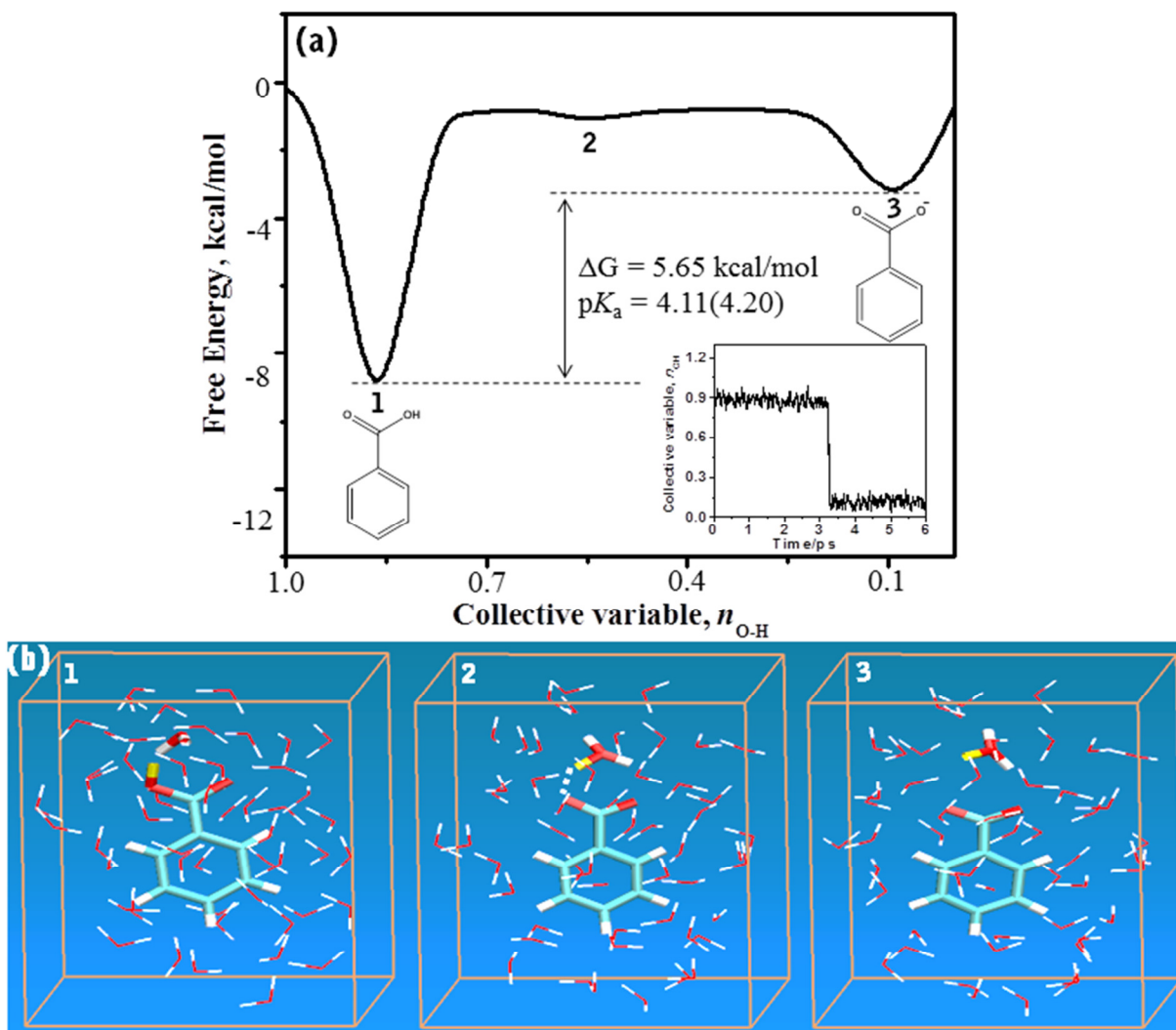


Figure 1 (a) The free-energy profile for the dissociation of benzoic acid. The difference in free energies of the dissociated and undissociated species, as well as the estimated pK_a value is indicated. The value in parentheses is the experimentally determined pK_a value of benzoic acid. The inset shows the evolution of the collective variable, n_{OH} , during the simulation (b) Snapshots along the trajectory at the locations indicated on Figure 1a. The proton being abstracted is highlighted in yellow and also the water molecules involved in the dissociation reaction.

right to the dissociated benzoate anion. In addition, a shallow minima is seen separating the reactant and product states. Snapshots along the trajectory at the locations indicated on Figure 1a are shown in Figures 1b (a movie file of configurations along the trajectory is provided in the Supplementary Information, Movie S1). It may be seen from the snapshots that the first minimum (labeled 1 in Figure 1a) corresponds to the solvated neutral benzoic acid. After 3.2 ps the proton is abstracted by a water molecule but the hydronium ion and the benzoate ion remain as a bound pair; the shallow minima (labelled 2) in the free energy profile corresponds to a contact-ion pair (the geometry of the contact-ion pair is described in the Supplementary Information, Figure S1). Subsequently the contact-ion pair breaks-up with the hydronium ion moving away to complete the dissociation process (the second minima, labeled 3, in Figure 1a).

It is possible to estimate the pK_a values from the free energy profiles in Figure 1a as the difference in the free energy of the dissociated and un-dissociated species, $pK_a = \Delta G / 2.303 RT$. The value of pK_a for benzoic acid estimated from the free energy profile (Figure 1a) 4.11 is comparable with the experimentally determined value, 4.20. The agreement with experiment is surprisingly good considering the nature of approximations used in this computation. A number of factors are responsible for the lack of accuracy in estimates of the absolute values of free energy obtained from a CPMD–metadynamics simulation. The main sources of error are the choice of the functional and secondly the errors associated with the metadynamics sampling technique. Quantum effects as well as the zero-point energy are also ignored in these calculations.³⁴ It may, however, be pointed out that here the pK_a values are estimated from the difference in free energy values associated with the neutral and dissociated species and consequently, there is a cancellation of errors. Errors associated with the use and choice of functionals are considerably reduced when one considers differences. The errors in the free-

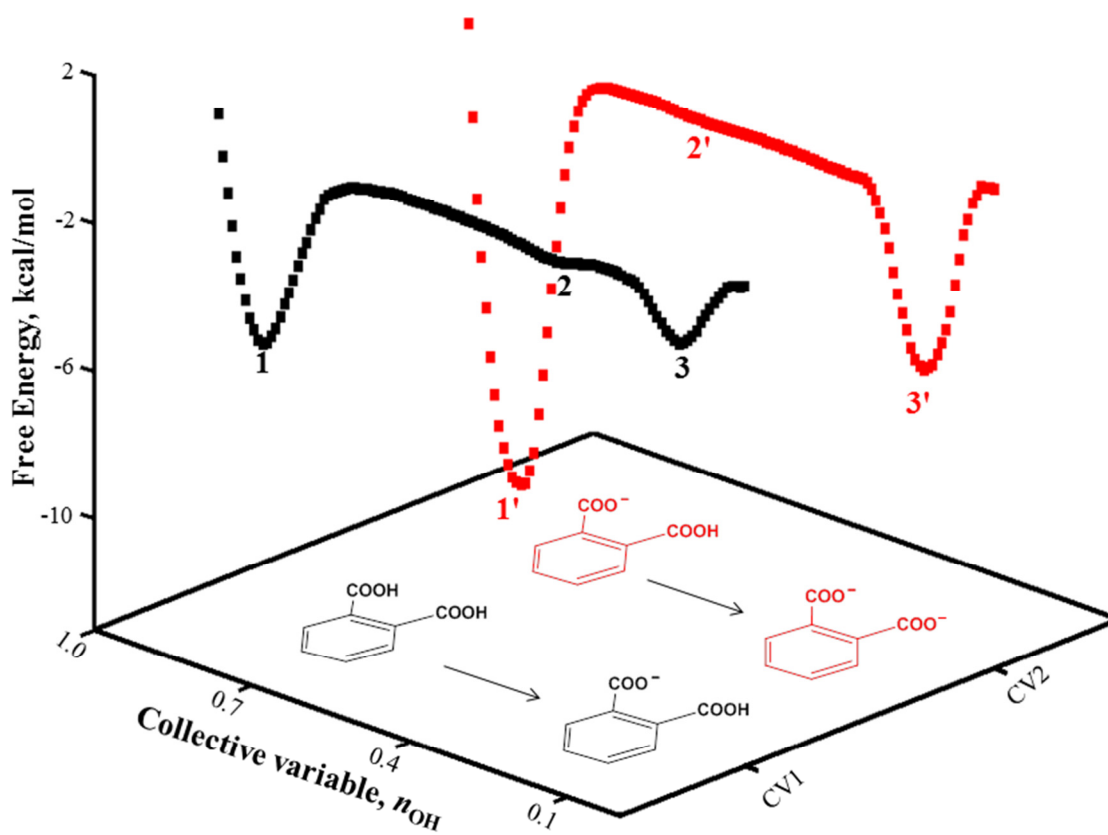


Figure 2 The free energy profiles for the first and second acid dissociation of phthalic acid. The collective variables CV1 and CV2 are defined by Equation 1.

energy values introduced by the metadynamics sampling depend essentially on the sampling parameters (see previous section) and are estimated to be of the order of $1.94 k_B T$, for the benzoic acid dissociation.³⁵ This error would be considerably reduced when the difference in free energy values are considered. It is therefore not surprising that the estimate of the pK_a value of benzoic acid, obtained as the difference in free-energy values leading to a cancellation of errors, is in good agreement with experiment. These results give us the confidence to attempt estimating pK_a values of acids with multiple ionizable groups present. In the following section

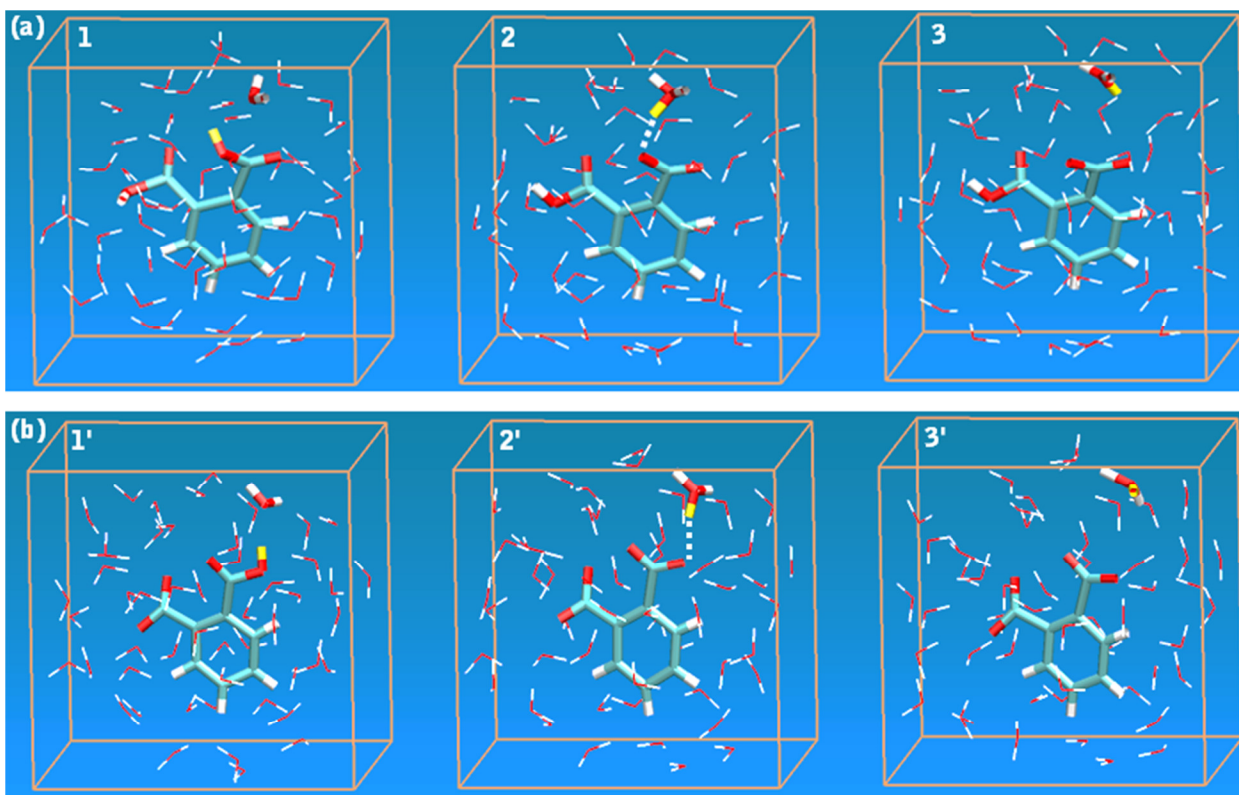


Figure 3 a) Snapshots for the first dissociation of phthalic acid at locations on the trajectory indicated in Figure 1. b) Snapshots for the second dissociation at locations on the trajectory indicated in Figure 1. The proton being abstracted is highlighted in yellow and also the water molecules involved in the dissociation reaction.

we use the *ab initio* metadynamics simulations to predict the first and second dissociation constants of phthalic acid and its isomers, isophthalic and terephthalic acids. One of the key features of the procedure adopted here is that all water molecules are treated explicitly at the same level of theory.

The free-energy profiles for the dissociation of phthalic acid and the subsequent dissociation of the singly charged phthalate ion are shown in Figure 2. As in the case for the dissociation of

Table 1 The predicted and experimental values of the first (pK_{a1}) and second (pK_{a2}) acid dissociation constants of phthalic acid, isophthalic acid and terephthalic acid.

Acid	Calculated		Experimental ^{36,37}	
	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
Phthalic	2.91	5.09	2.98	5.28
Isophthalic	3.34	4.22	3.46	4.46
Terephthalic	3.51	4.82	3.47	4.69

benzoic acid two well-defined minima are observed for both the first and second dissociation process; the minimum on the left corresponds to the un-dissociated species and that on the right to the dissociated ion. For both profiles a shallow minima is seen separating the reactant and product states corresponding to the formation of contact-ion pairs. Snapshots along the trajectory at the locations indicated on Figure 2 are shown in Figures 3a and b (a movie file of configurations along the trajectory is provided in the Supplementary Information, Movie S2 and S3). The dissociation behavior is similar to that of benzoic acid, the first minimum (labeled 1 in Figure 2) corresponds to the solvated neutral phthalic acid. After 2.5 ps the proton is abstracted by a water molecule but the hydronium ion and the phthalate ion remain as a bound pair that subsequently breaks-up to complete the dissociation process (the second minima, labeled 3, in Figure 2). The profile for the second ionization, the dissociation of the singly charged phthalate ion, is similar. The structure of the contact-ion pairs formed during the first and second dissociation reactions of phthalic acid are described in the Supplementary Information, Figure

S2. The CPMD-metadynamics simulations were also performed for isophthalic and terephthalic acids. The dissociation free-energy profiles for these isomers are similar to that shown in Figure 2 for phthalic acid (see Supplementary Information, Figure S3).

The first and second dissociation constants, pK_{a1} and pK_{a2} , of phthalic acid and its isomers were estimated from the difference in the free-energy values of the dissociated and un-dissociated hydrated species. The estimated and experimental pK_a values are shown in Table 1. It may be seen that the pK_a values predicted from the CPMD-metadynamics simulations are in good agreement with the experimental values. We would like to highlight the fact that not only do our calculations get the difference in the first and second pK_a values correct it can also predicts the more subtle difference and trends in the pK_a values of the isomers of phthalic acid.

CONCLUSIONS

In conclusion we have shown that quantum CPMD based metadynamics simulations can predict pK_a values of polyprotic weak acids with reasonable accuracy. The formalism treats all solvent water molecules explicitly while retaining conceptual simplicity. We use the distance-dependent coordination number of the protons bound to the carboxylic group as the collective variable to explore the free energy profile of the dissociation process. Two distinct minima corresponding to the dissociated and un-dissociated states of the acid are observed and the difference in their free energy values provides the estimate for pK_a , the acid dissociation constant. The method provides estimates of the pK_a value of benzoic acid comparable to experiment. We use phthalic acid and its isomers as the test system to establish that the metadynamics simulations are able to predict the trends and values of the first and second dissociation constants in good agreement with their

experimentally determined values. We believe that the procedures outlined here are of direct relevance to polyprotic acids where successive dissociation constants are not well separated and the experimental determination of the corresponding pK values difficult.

ASSOCIATED CONTENT

Supplementary Information Available: (Table S1) The number of water molecules and dimension of the simulation cell for the acid molecules investigated. (Movie S1) Movie files of the configurations along the dissociation trajectory of benzoic acid. (Figure S1) Structure of the contact-ion pair formed during the dissociation of benzoic acid. (Movie S2 & S3) Movie files of the configurations along the trajectory of the first and second dissociation of phthalic acid. (Figure S2) The O-H distances of the contact-ion pair formed during the first and second dissociation reaction of phthalic acid. (Figure S3) The free energy profiles for the first and second acid dissociation of (a) isophthalic acid (b) terephthalic acid. See <http://dx.doi.org/>.

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REFERENCES

- (1) K. S. Alongi and G. C. Shields, Theoretical Calculations of Acid Dissociation Constants: A Review Article, *Annu. Rep. Comput. Chem.*, 2010, **6**, 113-138.

- (2) E. Alexov, E. L. Mehler, N. Baker, A. M. Baptista, Y. Huang, F. Milletti, J. E. Nielsen, D. Farrell, T. Carstensen, M. H. Olsson, J. K. Shen,; J. Warwicker, S. Williams and J. M. Word, Progress in the Prediction of pK_a Values in Proteins, *Proteins*, 2011, **79**, 3260-3275.
- (3) J. Ho and M. L. Coote, A Universal Approach for Continuum Solvent pK_a Calculations: Are we there Yet?, *Theor. Chem. Acc.*, 2010, **125**, 3-21.
- (4) M. D. Liptak, K. C. Gross, P. G. Seybold, S. Feldgus and G. C. Shields, Absolute pK_a Determinations for Substituted Phenols, *J. Am. Chem. Soc.*, 2002, **124**, 6421-6427.
- (5) C. Lim, D. Bashford and M. Karplus, Absolute pK_a Calculations with Continuum Dielectric Methods, *J. Phys. Chem.*, 1991, **95**, 5610-5620.
- (6) C. P. Kelly, C. J. Cramer and D. G. Truhlar, Adding Explicit Solvent Molecules to Continuum Solvent Calculations for the Calculation of Aqueous Acid Dissociation Constants, *J. Phys. Chem. A*, **2006**, *110*, 2493-2499.
- (7) C. P. Kelly, C. J. Cramer and D. G. Truhlar, Aqueous Solvation Free Energies of Ions and Ion–Water Clusters Based on an Accurate Value for the Absolute Aqueous Solvation Free Energy of the Proton, *J. Phys. Chem. B*, 2006, **110**, 16066-16081.
- (8) C. P. Kelly, C. J. Cramer and D. G. Truhlar, SM6: A Density Functional Theory Continuum Solvation Model for Calculating Aqueous Solvation Free Energies of Neutrals, Ions, and Solute–Water Clusters, *J. Chem. Theory Comput.*, 2005, **1**, 1133-1152.

- (9) J. R. Pliego and J. M. Riveros, The Cluster-Continuum Model for the Calculation of the Solvation Free Energy of Ionic Species, *J. Phys. Chem. A*, 2001, **105**, 7241–7247.
- (10) J. R. Pliego and J. M. Riveros, Theoretical Calculation of pK_a Using the Cluster-Continuum Model, *J. Phys. Chem. A*, 2002, **106**, 7434-7439.
- (11) V. S. Bryantsev, M. S. Diallo and W. A. Goddard, III. Calculation of Solvation Free Energies of Charged Solutes Using Mixed Cluster/Continuum Models, *J. Phys. Chem. B*, 2008, **112**, 9709–9719.
- (12) J. Ho, Predicting pK_a in Implicit Solvents: Current Status and Future Directions, *Aust. J. Chem.*, 2014, **67**, 1441-1460.
- (13) C. H. Choi, S. Re, M. Feig and Y. Sugita, Quantum Mechanical/Effective Fragment Potential Molecular Dynamics (QM/EFP-MD) Study on Intra-Molecular Proton Transfer of Glycine in Water, *Chem. Phys. Lett.*, 2012, **539**, 218–221.
- (14) M. K. Ghosh, S. Re, M. Feig, Y. Sugita and C. H. Choi, Interionic Hydration Structures of NaCl in Aqueous Solution: A Combined Study of Quantum Mechanical Cluster Calculations and QM/EFPMD Simulations, *J. Phys. Chem. B*, 2013, **117**, 289–295.
- (15) M. K. Ghosh, N. Uddin and C. H. Choi, Hydrophobic and Hydrophilic Associations of a Methanol Pair in Aqueous Solution, *J. Phys. Chem. B*, 2012, **116**, 14254–14260.
- (16) N. Uddin, T. H. Choi and C. H. Choi, Direct Absolute pK_a Predictions and Proton Transfer Mechanisms of Small Molecules in Aqueous Solution by QM/MM-MD, *J. Phys. Chem. B*, 2013, **117**, 6269–6275.

- (17) J. G. Nelson, Y. Peng, D. W. Silverstein and J. M. J. Swanson, Multiscale Reactive Molecular Dynamics for Absolute pK_a Predictions and Amino Acid deprotonation, *J. Chem. Theory Comput.*, 2014, **10**, 2729–2737.
- (18) A. A. Hassanali, J. Cuny, V. Verdolino and M. Parrinello, Aqueous Solutions: State of the Art in *Ab initio* Molecular Dynamics, *Philos. Trans. R. Soc. A*, 2014, **372**, 20120482.
- (19) J. M. Park, A. Laio, M. Iannuzzi and M. Parrinello, Dissociation Mechanism of Acetic Acid in Water, *J. Am. Chem. Soc.*, 2006, **128**, 11318-11319.
- (20) M. Galib and G. Hanna, Mechanistic Insights into the Dissociation and Decomposition of Carbonic Acid in Water via the Hydroxide Route: An *Ab Initio* Metadynamics Study, *J. Phys. Chem. B*, 2011, **115**, 15024-15035.
- (21) J. G. Lee, E. Ascianto, V. Babin, C. Sagui, T. Darden and C. Roland, Deprotonation of Solvated Formic Acid: Car-Parrinello and Metadynamics Simulations, *J. Phys. Chem. B*, 2006, **110**, 2325-2331.
- (22) I. Ivanov, B. Chen, S. Raugei and M. L. Klein, Relative pK_a Values from First-Principles Molecular Dynamics: The Case of Histidine Deprotonation, *J. Phys. Chem. B*, 2006, **110**, 6365–6371.
- (23) M. Sulpizi and M. Sprik, Acidity Constants from Vertical Energy Gaps: Density Functional Theory Based Molecular Dynamics Implementation, *Phys. Chem. Chem. Phys.*, 2008, **10**, 5238–5249.
- (24) A. Laio and M. Parrinello, Escaping Free-Energy Minima, *Proc. Natl. Acad. Sci. U.S.A.*, 2002, **99**, 12562-12566.

- (25) M. Iannuzzi, A. Laio and M. Parrinello, Efficient Exploration of Reactive Potential Energy Surfaces Using Car-Parrinello Molecular Dynamics, *Phys. Rev. Lett.*, 2003, **90**, 238302-4.
- (26) A. Barducci, M. Bonomi and M. Parrinello, Metadynamics, *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, 2011, **1**, 826.
- (27) A. K. Tummanapelli and S. Vasudevan, Dissociation Constants of Weak Acids from Ab Initio Molecular Dynamics Using Metadynamics: Influence of the Inductive Effect and Hydrogen Bonding on pK_a Values, *J. Phys. Chem. B*, 2014, **118**, 13651–13657.
- (28) R. Car and M. Parrinello, Unified Approach for Molecular Dynamics and Density-Functional Theory, *Phys. Rev. Lett.*, 1985, **55**, 2471-2474.
- (29) CPMD, version 3.13.2, IBM Corp 1990–2008, MPI für Festkörper-forschung Stuttgart 1997–2001, <http://www.cpmc.org/>.
- (30) A. D. Boese, N. L. Doltsinis, N. C. Handy and M. Sprik, New Generalized Gradient Approximation Functionals, *J. Chem. Phys.*, 2000, **112**, 1670-1678.
- (31) S. Izvekov and G. A. Voth, Ab Initio Molecular Dynamics Simulation of Aqueous Proton Solvation and Transport Revisited, *J. Chem. Phys.*, 2005, **123**, 044505-9.
- (32) S. Grimme, Semiempirical GGA-Type Density Functional Constructed with a Long-Range Dispersion Correction, *J. Comput. Chem.*, 2006, **27**, 1787–1799.
- (33) N. Troullier and J. L. Martins, Efficient Pseudopotentials for Plane-Wave Calculations, *Phys. Rev. B*, 1991, **43**, 1993-2006.

- (34) M. Garcia-Viloca, C. Alhambra, D. G. Truhlar and J. Gao, Inclusion of Quantum-Mechanical Vibrational Energy in Reactive Potentials of Mean Force, *J. Chem. Phys.*, 2001, **114**, 9953-9958.
- (35) A. Laio, A. Rodriguez-Forteza, F. L. Gervasio, M. Ceccarelli and M. Parrinello, Assessing the Accuracy of Metadynamics, *J. Phys. Chem. B*, 2005, **109**, 6714-6721.
- (36) W.R. Maxwell and J. R. Partington, The Dissociation Constants of Some Polybasic Acids, *Trans. Faraday Soc.*, 1937, **33**, 670.
- (37) E. A. Braude and F. C. Nachod, *Determination of Organic Structures by Physical Methods*, New York, Academic Press, 1955.