

# Prediction of $pK_a$ Shifts without Truncation of Electrostatic Interactions: An Explicit Solvent Calculation for Succinic Acid

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The prediction of  $pK_a$  shifts from molecular models which incorporate solvent effects is a subject of renewed interest in biophysical chemistry. We have computed the difference in  $pK_a$ 's in succinic acid by an explicit solvent molecular dynamics simulation with Ewald summation and obtained results that are very close to the experimental value. The effects of conformational averaging are also considered by including both the trans and gauche conformers of succinic acid. For comparison, continuum electrostatic calculations for the  $pK_a$  shift in succinic acid were also carried out. The interaction free energy between the charged groups is overestimated when a standard small molecule value ( $\epsilon_i = 2$ ) is used to model the internal dielectric of the diacid. Although the explicit solvent simulations of  $pK_a$  shifts are more expensive in terms of computer time, the predictive power of these simulations has now progressed to the point where comparisons between explicit and continuum solvent models can reveal differences which have their true physical origin in the inherent molecularity of the surrounding medium.

## I. Introduction

The calculation of  $pK_a$  shifts is a long-standing problem.<sup>1–3</sup> Recently this field has been the object of renewed interest,<sup>4–16</sup> especially in the context of biological macromolecules. The accurate modeling of  $pK_a$  shifts in biomolecules is intimately related to a quantitative understanding of electrostatic features of biomolecular interactions. However, as the shifts result from the almost complete cancelation between between two large effects: the “direct” interactions among charged sites, i.e., in the absence of the solvent, and the solvent polarization contribution, their successful prediction is still a challenge. Obtaining  $pK_a$  shifts from molecular dynamics (MD) simulations in water which are in reasonable accord with experiment is very difficult, because of the delicate balance between the direct and indirect Coulomb interactions which is characteristic of strong dielectric shielding.

It is known that the incorporation of long-range effects, as in the Ewald summation method<sup>17–19</sup> when using periodic boundary conditions, results in good shielding for pure water<sup>20–24</sup> and ions in aqueous solution.<sup>25–29</sup> On the other hand, there is mounting evidence that their neglect leads to serious overestimation of the electrostatic interactions with consequent unphysical results.<sup>13,29–31</sup> Our own experience<sup>13,21,22,28,29</sup> has led us to consider the Ewald or equivalent method for handling the long-range interactions to be the best choice for problems where electrostatic interactions are dominant. The simulations reported in this letter have, except as noted, used the Ewald method to handle the electrostatic interactions.

Previous calculations have seldom taken into account the possibility that several conformers contribute to the observed shifts. This neglect is sometimes based on the argument that multiply charged states tend to select one out of the many possible conformers. However this fails to consider the contribution to the  $pK_a$  shift from the statistical distribution of states with different charges, and this neglect could in some cases result in significant errors. In this work, we predict the  $\Delta pK_a$  of a simple dicarboxylic acid, succinic acid, without

truncation of electrostatic interactions, including the effect of conformational averaging. Our choice of succinic acid to illustrate the problem of conformational averaging in predicting  $pK_a$ 's has been motivated by the fact that there are only two conformations to consider trans or gauche, and that recent calculations using the finite difference Poisson–Boltzmann (FDPB) method have yielded results that are not in accord with one another for succinic acid.<sup>11,14</sup>

The organization of this paper is as follows. In section II we derive the formulas for the  $pK_a$  shift using a linear response model for the free energy required to ionize the acid and also taking into account the contribution from different conformers. Although the derivation is general, some points are clarified by specializing to the dicarboxylic acid case. In section III the MD simulations are described in detail. We also describe some calculations based on continuum methods that were carried out for comparison. Finally, in section IV we present our results with a discussion of their significance for the accurate calculation of  $pK_a$ 's.

## II. Conformational Averages

All the calculations below used a model for succinic acid where the two oxygens of each carboxylic site had the same partial charges:  $-0.229e$  if the site is protonated and  $-0.729e$  if it is unprotonated. For a fixed conformation  $c$  let  $A^c(q_1, q_2)$  be the free energy of the system when the net charge on the oxygens of one carboxylic site is  $-2 \times 0.229e + q_1$  and that of the other site is  $-2 \times 0.229e + q_2$ . Thus  $q_i = 0$  corresponds to the protonation of site  $i$  and  $q_i = -1$  to its deprotonation. Using this notation the two  $pK_a$ 's are given by the equations

$$10^{-pK_{a1}} = 2 \frac{e^{-\beta A^c(0,-1)}}{e^{-\beta A^c(0,0)}} \quad (1a)$$

$$10^{-pK_{a2}} = \frac{1}{2} \frac{e^{-\beta A^c(-1,-1)}}{e^{-\beta A^c(0,-1)}} \quad (1b)$$

The factors of 2 and  $1/2$  in these equations appear because there

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are two possible states with one site protonated and one deprotonated, whereas there exists only one possibility if both sites have the same charge state.<sup>1</sup>

The free energy differences in eqs 1a and 1b can be related to the average electrostatic potentials by the identities

$$A^c(0,-1) - A^c(0,0) = -\int_{-1}^0 dq \langle V \rangle_{0,q} \quad (2a)$$

$$A^c(-1,-1) - A^c(-1,0) = -\int_{-1}^0 dq \langle V \rangle_{-1,q} \quad (2b)$$

where use was made of the symmetry relation

$$A^c(q_1, q_2) = A^c(q_2, q_1) \quad (3)$$

and  $\langle V \rangle_{q_1, q_2}$  is the mean value of the electrostatic potential at the site that is being titrated ( $q_2$ ) when the other site has charge  $q_1$ , averaged over the two oxygens comprising the site. Under the assumptions of linear response,<sup>32,33</sup> the average potential depends linearly on the charges:

$$\langle V \rangle_{q_1, q_2} = \langle V \rangle_{0,0} + Aq_1 + Bq_2 \quad (4)$$

where, by symmetry considerations,  $A = B$ , and therefore the integrations can be easily performed:

$$\int_{-1}^0 dq \langle V \rangle_{0,q} = \langle V \rangle_{0,0} - 1/2A = \langle V \rangle_{0,-1} + 1/2A \quad (5a)$$

$$\int_{-1}^0 dq \langle V \rangle_{-1,q} = \langle V \rangle_{0,0} - A - 1/2A = \langle V \rangle_{-1,-1} + 1/2A \quad (5b)$$

Upon subtraction the last term cancels and one arrives then at the equation

$$\Delta pK_a^c = pK_2^c - pK_1^c = \frac{1}{kT \ln 10} (\langle V \rangle_{-1,-1} - \langle V \rangle_{0,-1}) + 2 \log 2 \quad (6)$$

If more than one conformation is important in solution, eqs 1a and 1b need to be modified to give the observed  $pK_a$ 's:

$$10^{-pK_1} = 2 \frac{\sum_c e^{-\beta A^c(0,-1)}}{\sum_c e^{-\beta A^c(0,0)}} \quad (7a)$$

$$10^{-pK_2} = \frac{1}{2} \frac{\sum_c e^{-\beta A^c(-1,-1)}}{\sum_c e^{-\beta A^c(0,-1)}} \quad (7b)$$

so that

$$\Delta pK_a = \frac{1}{\ln 10} \ln \frac{(\sum_c e^{-\beta A^c(0,-1)})^2}{(\sum_c e^{-\beta A^c(0,0)}) (\sum_c e^{-\beta A^c(-1,-1)})} + 2 \log 2 \quad (8)$$

For succinic acid only the trans ( $t$ ) and gauche ( $g^+$ ,  $g^-$ ) conformations are important; then eq 8 can be written in terms of the individual  $pK^c$ 's using eqs 1a and 1b:

$$\Delta pK_a = \log \frac{(1 + 2e^{-\alpha} 10^{-\lambda})^2}{(1 + 2e^{-\alpha})(10^{-\Delta pK_a^t} + 2e^{-\alpha} 10^{-2\lambda} 10^{-\Delta pK_a^g})} \quad (9)$$

where

$$\alpha = \frac{1}{kT} (A^g(0,0) - A^t(0,0)) \quad (10a)$$

$$\lambda = pK_1^g - pK_1^t \quad (10b)$$

Note that eq 9 differs from the Boltzmann average of the  $\Delta pK_a$  (based on the doubly ionized state):

$$\frac{\Delta pK_a^t e^{-\beta A^t(-1,-1)} + 2\Delta pK_a^g e^{-\beta A^g(-1,-1)}}{e^{-\beta A^t(-1,-1)} + 2e^{-\beta A^g(-1,-1)}} \quad (11)$$

which can be rewritten as

$$\frac{\Delta pK_a^t + 2\Delta pK_a^g e^{-\alpha} 10^{-2\lambda} 10^{\Delta pK_a^t - \Delta pK_a^g}}{1 + 2e^{-\alpha} 10^{-2\lambda} 10^{\Delta pK_a^t - pK_a^g}} \quad (12)$$

From these considerations it follows that the observed  $\Delta pK_a$  is not just given by the contribution from the predominant (trans in this case) conformation in the doubly ionized state, as would be predicted by eq 12. This argument ignores the fact that both conformations may be populated in the singly ionized and doubly protonated states.

As discussed below, from MD simulations with explicit solvent we obtain the estimates  $\Delta pK_a^t \approx 1.1$  and  $\Delta pK_a^g \approx 3.8 \gg \Delta pK_a^t$ . Taking into account both gas-phase and solvation contributions (see discussion below), both  $\alpha$  and  $\lambda$  are close to zero. We can therefore ignore the term in eq 9, arising from the doubly ionized gauche state because it is almost 4 kcal/mol less stable than the corresponding trans state, and simplify eq 9 to

$$\Delta pK_a = \Delta pK_a^t + \log \frac{(1 + 2e^{-\alpha} 10^{-\lambda})^2}{1 + 2e^{-\alpha}} \quad (13)$$

### III. Methods

To compute  $\Delta pK_a^t$  and  $\Delta pK_a^g$ , molecular dynamics (MD) simulations using the program IMPACT<sup>34</sup> were carried out on two systems, each comprised of one succinic acid solute immersed in a bath of 1102 SPC<sup>35</sup> water molecules. One system had the solute fixed in the trans conformation and the other had it in the gauche conformation. The initial coordinates of succinic acid in the trans conformation were obtained from the Cambridge Crystallographic Database.<sup>36,37</sup> The gauche conformation was obtained through the appropriate 60° rotation. The equations of motion were integrated using Andersen's RATTLE<sup>34,38</sup> algorithm with a time step of 1.5 fs. The solutes were held fixed during the simulations; this constraint was enforced by setting the forces on each solute to zero at each time step. The density of the systems was approximately 1 g/cm<sup>3</sup>, corresponding to a cubic cell of length 32.0 Å. Periodic boundary conditions were consistently implemented, including the use of the Ewald summation method<sup>17,19,39,40</sup> for the long-range electrostatic interactions. A nonbonded list was regenerated every 7.5 fs. The Ewald convergence parameter  $\alpha$  was set to 5.5/ $L$ , where  $L$  is the cubic cell length; the reciprocal space portion of the Ewald potential was evaluated using  $k = 5$ , with  $k^2 < 14$ . The nonelectrostatic part of the solute-water potential was a Lennard-Jones 6-12 potential with parameters given by Weiner et al.<sup>41</sup> The temperature was fixed at 298 K using Berendsen's scaling method with a relaxation time of 0.01 ps.<sup>42</sup> For each simulation the systems were allowed to equilibrate for 75 ps, followed by 405 and 120 ps production

**TABLE 1: Atomic Partial Charges Used for Succinic Acid in Its Neutral Form**

atom type	$q$ (au)
C(H <sub>2</sub> )	-0.398
H	0.071
C(OO)	0.714
O	-0.229

**TABLE 2:  $\Delta pK_a$  for the Trans and Gauche Conformations of Succinic Acid Obtained from the Explicit Solvent Simulations with Ewald Summation<sup>a</sup>**

Trans (MD)	Gauche (MD)	Conformational Average (MD)	Experimental
1.1 ± 0.3	3.8 ± 0.3	1.6 ± 0.3	1.43

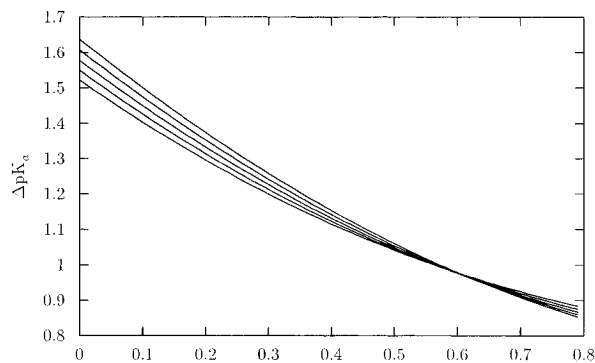
<sup>a</sup> The predicted conformational average  $pK_a$  shift calculated with  $\alpha = \lambda = 0$ . The experimental value is taken from Westheimer and Shookhoff<sup>3</sup> but has been adjusted for the statistical factor.

runs for the trans and gauche succinic acid solutions respectively. Configurations were stored every 120 fs for subsequent analysis.

For each conformation of the solute, trans and gauche, two simulations were performed that differed in the charge state of the solute. In simulation A all the titratable oxygens had a charge of  $-0.729e$ , corresponding to the deprotonated state; in simulation B one of the two titratable sites was protonated, increasing the oxygens partial charges to  $-0.229e$ , while the other remained deprotonated. The atomic partial charges were obtained from the AMBER force field used by IMPACT,<sup>34</sup> with slight modifications to obtain the correct net charge of the molecule. The partial charges used in the simulations are shown in Table 1.

#### IV. Results and Discussion

From the simulations described above the corresponding  $pK_a$  shifts were computed using eq 6. The results are displayed in Table 2. As the computed  $\Delta pK_a^t$  is much smaller than  $\Delta pK_a^g$ , we can use the simplified eq 13 to obtain the observed  $\Delta pK_a$ . To estimate the values for the parameters  $\alpha$  and  $\lambda$  in eqs 10a and 10b, both vacuum and solvent contributions to the free energy differences between the trans and gauche states are needed. The vacuum contributions for both the neutral and singly ionized states are taken to be equal to the difference in energy of the two conformations using Gaussian 92<sup>43</sup> at the HF/6-311G\*\* level, which results in the gauche conformation being more stable by about  $0.98kT$  (the neutral state) and  $1.42kT$  (the singly ionized state). The solvent contribution to the free energy difference of the neutral states between both conformations was evaluated using the free energy perturbation method.<sup>44,45</sup> For each case the molecule is initially rendered nonpolar and subsequently the Lennard-Jones cavity is disappeared. In this case, the trans conformation is more stable by about  $(0.7 \pm 0.2)kT$ . The solvent contribution to the free energy difference of the singly ionized conformations was related to the average electrostatic potential. This method is analogous to the one described earlier for the calculation of  $pK_a$  shifts assuming linear response behavior. For the solvent contribution, the trans conformation is also more stable by about  $(2.0 \pm 0.8)kT$ . Taken together these calculations suggest that both  $\alpha$  and  $\lambda$  are close to zero. In Figure 1 we present a plot of the observed  $pK_a$  as a function of both parameters: the abscissa is  $\lambda$  and the different curves correspond to  $\alpha$  varying between  $-0.2$  and  $+0.2$ . In this region the results are not very sensitive to  $\alpha$ . The Boltzmann average, eq 12, which neglects the effect of the nearly equal gauche and trans populations of the fully protonated state, gives, over the same range, an essentially constant value of  $\Delta pK_a^t$ .



**Figure 1.**  $\Delta pK_a$  from eq 13 as a function of  $\alpha$ , the free energy difference between the fully protonated gauche and trans conformations, and  $\lambda$ , the difference in the first  $pK_a$ 's. The abscissa corresponds to  $\lambda$  and the different curves to  $\alpha$  varying from  $-0.2$  to  $0.2$ . By contrast, the Boltzmann average in eq 12 predicts an essentially flat line at 1.1  $pK$  units.

**TABLE 3:  $\Delta pK_a$  for Each Conformer Obtained with the Continuum Dielectric Method<sup>a</sup>**

	trans	gauche	conformational average
this group	1.6	2.9	2.1
Rajasekaran et al. <sup>14</sup>	1.4		1.9
Potter et al. <sup>11</sup>	2.4		2.9

<sup>a</sup> The result of Rajasekaran et al. has been adjusted by adding the statistical factor  $2 \log 2$ . The average column reports the conformational average for  $\alpha = \lambda = 0$  and  $\Delta pK_a^g = 2.9$ . Note that  $\alpha < 0$  produces still larger values.

The effect of  $\lambda$ , that is, the difference in population of the singly charged state, is more pronounced. Near the lower limit of the range we approach the experimental value of 1.43  $pK$  units, while close to the upper limit the predicted  $\Delta pK_a$  drops to about 0.9 units. It is interesting to note that even if both singly and doubly charged states are populated only by the trans conformation, the predicted  $pK_a$  is not simply that of the trans conformation if for the fully protonated diacid both the trans and gauche conformations are about equally populated.

We have also computed the  $\Delta pK_a$ 's for each conformer within the continuum dielectric model using the program DelPhi.<sup>46</sup> The initial conformations, atomic partial charges, and van der Waals radii of the trans and gauche solutes were the same as used in the MD simulations. The molecule was initially mapped onto a  $65 \times 65 \times 65$  point cubic lattice with a resolution of 2 grids/Å, and a two-step finite-difference focusing with resolutions of 6 and 9 grids/Å was employed.<sup>46</sup> The solvent was treated as a continuum of dielectric constant of value 80. A dielectric constant of 2 was assigned to the solute molecule. The Richards probe-accessible surface definition, with a radius of 1.4 Å, was used to determine the extent of the low dielectric region.<sup>47</sup>

Table 3 compares our results with those of Potter et al.<sup>11</sup> and Rajasekaran et al.<sup>14</sup> The conformational averages were performed with  $\alpha = \lambda = 0$ ; for any other value of  $\alpha$  and  $\lambda$  eq 9 can be used to obtain the corresponding value. Our calculation used the same charge model as the explicit solvent simulations. The conformational average results used our value for the  $\Delta pK_a^g$  since this was not reported in the other references. The spread in values is probably due to differences in the solute model as well as in the treatment of the dielectric boundary. They all yield, however, too large a  $\Delta pK_a$  if the conformational average is performed as above. An increase in the choice of the internal dielectric of the diacid would of course increase the screening between the charges and decrease the predicted shift.

## V. Conclusions

We have performed all-atom molecular dynamics simulations of succinic acid in aqueous solution using the Ewald summation method. Our choice was based on previous observations that truncating the electrostatic forces leads to unphysical low-shielding behavior.<sup>13,29–31</sup> We obtain the  $pK_a$  shifts in both the trans and gauche conformations. With the Ewald potential the explicit solvent molecular dynamics simulations leads to a predicted  $pK_a$  shift that lies close to the experimental value. The shifts obtained for both the trans and gauche conformations need to be combined since for the neutral and singly ionized forms of the diacid, both conformers are populated. To combine the shifts one must do a consistent treatment of all conformations for all protonation states, rather than a Boltzmann average of only one of the protonation states. For the final evaluation, the free energy differences between the conformers are needed for both the neutral and singly charged species.

The prediction of  $pK_a$  shifts in the condensed phase is a computational problem which demands extremely high accuracy. The reason for this is that the differential environmental effects of interest are typically of the order of  $\approx kT$  in free energy units ( $\approx 1 pK_a$  unit) while the absolute contribution to the  $pK_a$  due to the polarization of the medium by the ionization process is of the order of 100 kcal/mol. In our experience, it has not been possible previously to achieve this level of accuracy with free energy simulations in explicit solvent using truncated potential models, when electrostatic effects predominate. The level of accuracy we have been able to achieve in the present explicit solvent simulations of the  $pK_a$  shift in succinic acid using the Ewald potential is at least comparable to the corresponding accuracy we obtained using the continuum solvent model. Although the explicit solvent simulations of  $pK_a$  shifts are more expensive in terms of computer time, the predictive power of these simulations has now progressed to the point where comparisons between explicit and continuum solvent models can reveal differences which have their true physical origin in the inherent molecularity of the surrounding medium.

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