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Deprotonation of a Histidine Residue in Aqueous Solution Using Constrained Ab Initio Molecular Dynamics

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The concept of acidity is fundamental to physical and structural chemistry. A great number of chemical, biological, and industrial processes either involve acid/base reactions or are dependent on the protonation state of the chemical species. Therefore, the topic of accurate pK_a determination has received a great deal of attention both experimentally and theoretically.[1,2] Despite these efforts, the calculation of pK_a's in condensed phase medium is fraught with difficulties. A first principles quantum mechanical approach with explicit inclusion of the solvent is imperative to achieve quantitative understanding of dissociative processes without employing a priori assumptions. In that respect, the major advantage of using the Car−Parrinello (CPMD) methodology[3] is that it limits reliance on empirically determined potentials and thus allows for an accurate description of bond breaking and formation.

In the present study, we have used constrained ab initio molecular dynamics (AIMD) to investigate the deprotonation of a histidine residue in aqueous solution. Histidine plays an important role in many biochemical processes such as proton transfer at enzymatic active sites.[4a] Its tautomeric equilibrium is also of great biophysical importance.[4b,c] In this communication, we have focused on calculating potentials of mean force for a number of deprotonation reactions. We have related the obtained free energy differences between the protonated and the deprotonated forms to the experimental pK_a values. Additional analysis of the structural, electronic, and dynamical transformations along the selected reaction paths was performed. The results highlight the importance of the interplay between electrostatic solvation, chemical and hydrogen bonding, as well as dynamical finite temperature effects in determining acid/base properties.

AIMD simulations have been carried out on aqueous solutions containing either L-histidine or capped histidyl residues. All simulations were performed with the program CPMD.[5] The gradient corrected exchange-correlation functional of Hamprecht et al.,[6] denoted HCTH, was adopted for the present study because it has been shown to yield significantly improved energetics as compared to the popular Becke−Lee−Yang−Parr (BLYP) functional.[7] To account for the valence−core interactions, we employed norm-conserving Troullier−Martins pseudopotentials.[8] The simulation cell contained one histidine residue plus 49 water molecules and was periodically replicated in three dimensions. Its size (11.76 Å) was determined from experimental density data.[9a] The valence electronic wave functions were expanded in a plane wave basis set with an energy cutoff of 70 Ry. All hydrogen nuclei were replaced by deuterium. A fictitious electronic mass of 900 au was selected which permitted the integration of the equations of motion with a time step of 0.165 fs. The simulations were performed in the canonical ensemble.

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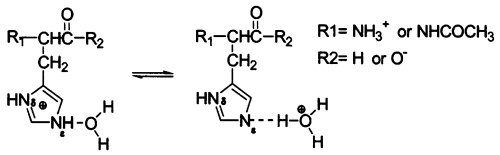
On the basis of standard electrochemical titration data,[1d,9b] histidine has three measurable pK_a's with values of 1.70, 6.04, and 9.12, respectively. We have focused only on the second stage of dissociation due to its biological relevance. To investigate the tautomeric equilibrium of histidine, we have carried out deprotonation from both the Nϵ and the Nδ positions of the imidazole ring. We have also attempted to assess the role of electrostatic interactions with the carboxylate and amino groups of the free L-histidine by studying a capped analogue. The deprotonation reaction is represented schematically below:



The dissociation of histidine is clearly a rare event on the time scale of ab initio CPMD. Therefore, to study the process, we have selected a reaction coordinate and constrained its value in a range suitable to effect proton transfer. In particular, the N*−H* distance between one of the imidazole nitrogens and its neighboring hydrogen ($\xi = |r_{N^*} - r_{H^*}|$) was varied between 1.1 and 1.8 Å with an increment of 0.05 Å. For each of the 15 values, we have obtained constrained trajectories starting from well equilibrated unconstrained configurations. After reequilibrating for at least 1.0 ps, the properties of interest were averaged until convergence (3 to 4 ps) as evidenced by the running averages of the constraint force. As a result, the total simulation time amounted to more than 200 ps. To eliminate the possibility of bias due to the choice of a simple distance constraint, the results were verified by applying the coordination constraint of M. Sprik.[2a−c] The relative free energy between states ξ_0 and ξ_1 can be evaluated using the expressions:

$$\Delta F = -\int_{\xi_0}^{\xi_1} f_{\xi'} d\xi' \qquad f_{\xi'} = \frac{\langle Z^{-1/2}[\lambda - kTG]\rangle_{\xi'}}{\langle Z^{-1/2}\rangle_{\xi'}}$$

where f_ξ is the average force of constraint; k is the Boltzmann constant; T is the temperature in K; and Z and G are weight and correction factors.[2c] In the case of a distance constraint, the second equation reduces to a simple ensemble average of the Lagrange multiplier λ: $f_{\xi'} = \langle\lambda\rangle_{\xi'}$.

Thermodynamic integration[2] yields the free energy profiles plotted in Figure 1. It is clear from the plot that the dissociation proceeds in several stages. First, the polar N*−H* bond is extended similar to normal vibrational stretching, and the free energy curve is convex. The bond transformation (conversion of N*−H* from chemical to hydrogen bond) takes place for values of ξ from about 1.2 to 1.35 Å. At 1.4 Å, the proton is already completely transferred to the O* atom. Any further increases in the value of ξ correspond

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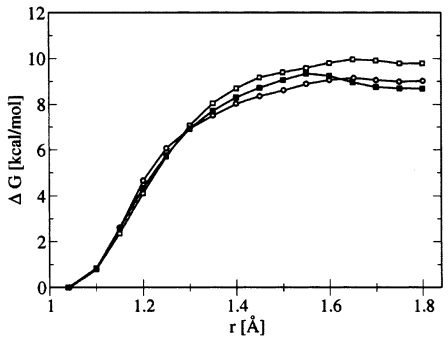

Figure 1. Potentials of mean force for the deprotonation of (i) L-histidine from the Nδ (open square line) and (ii) Nϵ (filled square line) positions using distance constraints. The open circle line corresponds to an analogous dissociation profile from the Nϵ position of a capped residue.

to stretching a hydrogen bond and are therefore accompanied by much smaller energetic effects. As a result, the free energy curves level off after this point. From about 1.5 Å, it becomes possible for the hydronium ion to proton transfer to neighboring water molecules. Indeed, visual inspection of the trajectories confirms that proton transfer events are frequent but generally do not lead to complete separation of the hydronium until the transition state ($\xi = 1.6-1.65$ Å) when the constraint force changes its sign. From that point on, the products are formed, and the hydronium ion eventually diffuses in solution. For all three cases, the reverse proton transfer barrier is either very small or essentially absent. Therefore, the reverse reaction is primarily diffusion limited. It should be noted, however, that due to the inevitable limitations of the density functional[2b,6] and the small system dimensions, the determination of barrier heights may be less reliable. As pointed out by Sprik,[2b] a clear distinction between ΔG and ΔG^* may not be possible in the case of ab initio CPMD. With these caveats in mind, we proceed to make a comparison with the experimental free energy differences. A simple and reliable way to estimate the change in free energy for dissociation of a weak acid is given by the following equations:

$$\Delta G = \Delta G^\circ + 2.303RT \log([\mathrm{Im}][\mathrm{H}^+]/[\mathrm{ImH}^+])$$
$$\Delta G^\circ = 2.303RT\mathrm{p}K_\mathrm{a}$$

Because concentrations in our systems (1.02 M) have been chosen close to the standard concentration, the relevant value to compare with is ΔG°. Using the above formulas, we have determined that the estimated value of 8.4 kcal/mol compares favorably with our calculated free energy differences of 8.7 and 9.8 kcal/mol for the deprotonation of L-histidine from the Nϵ and Nδ positions, respectively. For the capped analogue, a calculated free energy difference of 9.05 kcal/mol should be compared to the experimental estimate of 9.56 (based on the pK_a of *N*-acetyl-L-histidine $\approx$ 6.96). The result for dissociation initiated by a coordination constraint seems consistent with the one obtained with a linear constraint within the limits of statistical uncertainty ($\sim$1 kcal/mol). The Gibbs free energy of 1.1 kcal/mol associated with the tautomeric equilibrium of histidine corresponds to a tautomeric form ratio of 0.16 in good agreement with the experimental estimate of $\sim$1:4.[1d]

A unique advantage of AIMD is that it allows a detailed microscopic analysis of bond breaking and formation from the point of view of quantum chemistry. In particular, the electron localization function (ELF) of Becke and Edgecombe[10] is a convenient tool for monitoring the bond breaking process. Along the reaction pathway, ELF varies parametrically with the nuclear configuration, and the transformations in bonding are manifested in the appearance and disappearance of local maxima. Several mechanisms may be

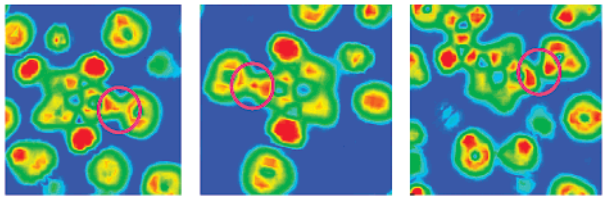

Figure 2. Contour plots of the electron localization function (ELF) for three values of ξ: (i) 1.3 Å; (ii) 1.35 Å; and (iii) 1.6 Å. The N−H bond of interest is highlighted with a red circle.

invoked to describe the proton transfer process. In a purely ionic picture, the proton is expected to cross the separatrix of two basins in the localization function, leaving the total number of basins unchanged. By contrast, a covalent dissociation entails the breaking of an NH bond and formation of an OH bond either simultaneously or in two sequential steps. In the first case, there would be no variation in the number of basins, whereas, in the second case, the appearance of a new basin corresponding to an intermediate is observed. From Figure 2, panel ii, it is evident that the proton transfer in our case is not a fully concerted process but seems to happen in two steps with an intermediate structure at $\xi = 1.35$ Å. However, the process is not fully covalent as evidenced by an analysis of the position and spread of the Wannier function centers (not shown).

In conclusion, we would like to point out that the ab initio molecular dynamics method has made possible unprecedented insight into the mechanisms of complex chemical processes in condensed phase systems. Our results clearly demonstrate that the calculation of free energies of reactions from first principles is possible. Future work will examine applications of the Car−Parrinello methodology and novel methods for finding reaction paths[11a,b] to the study of the catalytic activity of hydrolytic enzymes.

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