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Molecular Dynamics Potential of Mean Force Calculations: A Study of the Toluene−Ammonium π-Cation Interactions

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Abstract: We have examined the interaction between the ammonium cation and the aromatic face of toluene in water, by means of potential of mean force (PMF) calculations. Considering that (i) typical two-body additive molecular mechanical models cannot represent the energetics of π-cation interactions accurately and (ii) employing nonadditive force fields increases the computational effort significantly, we have incorporated a short-range "10−12" term in our potential function, ensuring that the magnitude of the attraction between ammonium and toluene reproduces the value estimated from high-level quantum mechanical calculations. Interestingly, the PMF curve generated in water clearly demonstrates that association is favorable in a polar aqueous medium, with a minimum of the free energy equal to *ca*. 3 kcal/mol, and an association constant of 6.5 M^{-1}—consistent with experimental data on related π-cation systems. This association appears to be even stronger when the approach of ammonium toward the toluene ring is axially constrained, hence indicating that, in addition to non-negligible entropic effects, the magnitude and the directionality of ammonium−aromatic interactions might be intimately related. A comparison of the free energy profiles obtained in a *vacuum* and in water suggests that "contact" configurations should be stabilized in nonpolar environments. This observation concurs with the analysis of Phe−Lys interactions in several protein crystal structures.

1. Introduction

Twenty years ago, Kier and co-workers[1,2] observed that, in addition to the negatively charged amino acid side chains Asp$^-$ and Glu$^-$, the aromatic residues (*e.g.* Phe, Trp, and Tyr) in an active site might also take part in the binding of the onium group of a given substrate. It has been subsequently shown, with both experimental measurements in the gas phase and STO-3G ab initio calculations,[3] that the prototypical ammonium ion forms a strong complex with benzene—the theoretical calculations revealing that the most stable dimer corresponds to a bidentate complex where the nitrogen and the center of the benzene ring are *ca*. 3.0 Å apart.

At the same time, these interactions, prosaically called "π-cation" interactions, between protonated amines and aromatic residues have also been recognized as favorable stabilizing elements in proteins. From a statistical geometrical analysis of X-ray protein structures, Burley and Petsko[4] and Singh and Thornton[5] noticed that the positively charged amino groups were preferentially localized near the π-electron cloud of the aromatic ring of the Phe, Trp, or Tyr side chains.

π-Cation interactions are also likely to play a key role in the formation of complexes between quaternary ammonium systems and small synthetic or protein receptors,[6,7] thereby providing an additional force for intermolecular recognition. An "aromatic triggering mechanism" has been proposed, reflecting an emerging trend which suggests that, in these receptors, the positively charged ammonium can be surrounded by aromatic compounds. This motif has received recent support with regard to amine-based neutrotransmitters[8−12] and G-protein coupled receptors.[13] The underlying hypothesis has been strengthened by a recent statistical analysis of the interactions between phenyl rings and $R-N^+(CH_3)_3$ cations in X-ray structures extracted from the Cambridge Structural Database.[10] Additional studies carried out on similar protein receptors have suggested that the magnitude of π-cation interactions can be substantial, even in aqueous

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media, where a competition between the onium−water and onium−aromatic interactions may exist.[11]

The essential stabilization effects between onium cations and neutral aromatic groups are now interpeted as a directional $1/\mathbf{r}^3$ charge-quadrupole attraction[14] between a positive charge and the "anionic" face of the aromatic ring,[15,3,16] supplemented by dispersion attraction and polarization.[17,18] Interestingly enough, recent ab initio calculations on the archetypical benzene−ammonium dimer have indicated that the bidentate complex is slightly energetically favored over those geometries where a single N−H bond points toward the π cloud (*i.e.*, monodentate motif), or where three N−H bonds are equidistant from the center of the ring (*i.e.*, tridentate motif).[11]

In this study, we quantitatively determine the magnitude of the π-cation interaction between toluene and ammonium embedded in an explicit solvent, by means of potential of mean force (PMF) calculations. Since it has been observed that adding an organic solvent to an aqueous medium diminishes the binding of the onium group to the aromatic ring,[11] we calculate the free energy profile *in vacuo* and in an aqueous medium, in order to determine the influence of the surroundings on the π-cation interaction.

2. Methods: Computational Details

The emphasis in the present work is on the computation of free energy profiles, characterizing the approach of two solutes as a function of an intermolecular distance. Among the most frequently utilized methods for evaluating the free energy change between two given points on such profiles are the "umbrella sampling" technique,[19−22] free energy perturbation[23−25] (FEP), and thermodynamic integration[24−27] (TI). While the two latter alternative computational methods have proven to yield results of comparable quality, TI has appeared to constitute, under certain circumstances, a slightly more efficient protocol for the derivation of PMF.[24−28] This approach, which will be employed herein, relies on the following master equation:

$$\Delta G = \int_0^1 \left\langle \frac{\partial H(\mathbf{r};\lambda)}{\partial \lambda} \right\rangle_\lambda \mathrm{d}\lambda \qquad (1)$$

where the potential energy function is of the form[29,30]

$$H_{\text{total}}(\mathbf{r}) = \sum_{\text{bonds}} k_r(r-r_0)^2 + \sum_{\text{angles}} k_\theta(\theta-\theta_0)^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1+\cos(n\phi-\omega)] + \sum_{i<j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_iq_j}{\epsilon R_{ij}}\right] + \sum_{\substack{\text{H-bonds}\\ \pi\text{-cation}}}\left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right] \qquad (2)$$

We have demonstrated recently that the TI method generally leads to a better convergence of the free energy when the integrand is evaluated at a limited number of points involving extensive sampling[31,28] (assuming a reasonably slowly varying ΔG *versus* λ curve), rather than at numerous λ points with less sampling. In the PMF simulations reported here, no more than 100 points were employed to change from 8.0 to 2.5 Å the distance between nitrogen and the center of the aromatic ring (see Figure 1). At each λ point, this distance was kept fixed using an appropriate holonomic constraint.[32] Because experimental X-ray observations suggest that the positively charged amino group approaches the "anionic" face of the ring axially,[4] we have defined six additional angular constraints $\{\mathrm{N, D, C}_i\}$ set to the fixed value of 90.0° (see Figure 1), preventing the ammonium ion from drifting around the 6-fold C_6 symmetry axis of the aromatic ring. The λ-dependence of the constrained distance separating the nitrogen atom from the noninteracting centroid of the toluene ring is introduced in the TI formulation *via* the potential force (PF) method,[31] allowing a fast and accurate determination of the holonomic constraint contribution to the free energy, $\partial H^{\text{constr}}(\mathbf{r};\lambda)/\partial\lambda$.

In the case of the PMF simulation in water, at each λ-point of the integrand, 20 ps of classical constant pressure molecular dynamics (MD) was used to generate the statistical ensemble[33] over which the quantity $\partial H(\mathbf{r};\lambda)/\partial\lambda$ was averaged. This amount of MD sampling is decomposed into 5 ps of equilibration and 15 ps of data collection. Employing 50 windows, the overall simulation time corresponds to 1 ns. It is necessary that the molecular system be equilibrated thoroughly in order to ensure that the system does not lag the Hamiltonian when data collection starts.[28,34−36]

All the PMF computations were carried out using the molecular simulation package GIBBS/AMBER 4.1[37] and the standard Weiner *et al.*[30] and Hagler *et al.*[38] van der Waals parameters, supplemented by atomic charges calculated using a least-squares fit to the quantum mechanically derived potential[39,40] (see Table 1). The standard AMBER mixing rules were employed for the Lennard-Jones contribution to the free energy, *i.e.*, $r_{ij}^* = (r_{ii}^* + r_{jj}^*)/2$ and $\epsilon_{ij} = (\epsilon_{ii}\epsilon_{jj})^{1/2}$. The geometries of the two solutes were optimized at the Hartree−Fock (HF) level of approximation, using the split-valence 6-31G** basis set[41] (see Figure 1), and the point charge distributions were determined from the corresponding wave functions. Except for the aromatic torsions,[42,43] standard AMBER force constants[30] were employed to evaluate the intramolecular interactions. It is worth noting that the molecular

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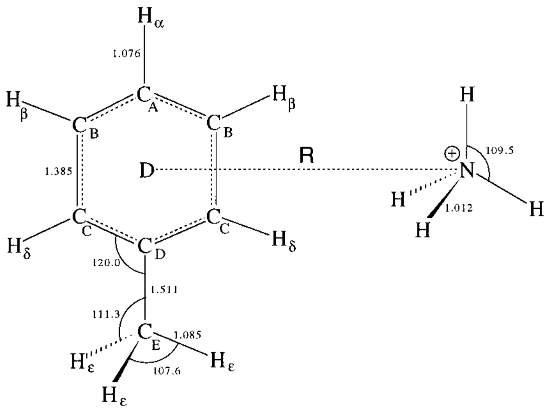

Figure 1. Geometrical parameters used for the molecular simulations. All bond lengths in Å and valence angles in deg. A pseudo-atom "D", located at the center of the ring, is used to define the constrained distance "*R*" for the potential of mean force calculations.

Table 1. Nonbonded Parameters Used in the Molecular Simulations

molecule[a]		charges (ecu)	Lennard-Jones parameters r^*_{ii}(Å)	ϵ_{ii}(kcal/mol)
toluene	C_A	−0.189	1.8500	0.1200
	H_α[b]	0.151	1.3750	0.0380
	C_B	−0.128	1.8500	0.1200
	H_β[b]	0.147	1.3750	0.0380
	C_C	−0.279	1.8500	0.1200
	H_γ[b]	0.158	1.3750	0.0380
	C_D	0.353	1.8500	0.1200
	C_E	−0.574	1.8000	0.0600
	H_ϵ[b]	0.154	1.3750	0.0380
	D	0.000	0.0000	0.0000
NH_4^+	N	−0.911	1.8500	0.0800
	H	0.478	1.0000	0.0200
H_2O (TIP3P[52])	OW	−0.834	1.7680	0.1520
	HW	0.417	0.0000	0.0000

[a] See Figure 1. [b] Modification of the original AMBER all-atom force field.[38]

mechanics model adopted to describe the solutes has proven to lead to absolute free energies of hydration in reasonable agreement with experimental data.[44,45] In the particular case of toluene and ammonium, we obtained, respectively, −1.46[46] and −81.03[44] kcal/mol (−101.31 kcal/mol if a Born[47] correction term is incorporated to take into account the long-range electrostatic effects), to be compared to the experimental values of −0.88[48] and −78.6[49] kcal/mol. While the accord with experiment falls roughly within "chemical accuracy" for toluene, the Born corrected free energy of hydration of ammonium is significantly overestimated.

In fact, the example of ammonium perfectly illustrates the shortcomings of molecular mechanics calculations conducted in the framework of pure two-body additive models. Recently, Meng *et al.*[50] have demonstrated that an accurate reproduction of the hydration free energies of ions is possible under the *sine qua non* condition that nonadditive effects be taken into account appropriately. Unfortunately, free energy calculations involving explicit polarizabilities are extremely CPU demanding (*i.e.*, increasing 4- to 5-fold the cost of a standard free energy calculation) and, in spite of the continuous decrease of the computer price/performance ratio, still remain very expensive in the context of PMF simulations. In the present work, we consider two different additive models to represent the π-cation interaction between toluene and ammonium, *viz.* a standard one, using the aforementioned intra- and intermolecular parameters, and a modified one, presenting all the features of the latter, but including a specific short-range 10−12 potential—between the nitrogen of ammonium and the carbon atoms constitutive of the aromatic ring—in order to reproduce semiquantitatively both the energetics and the equilibrium geometry obtained at the ab initio MP2/6-31G**//HF/6-31G** level of approximation. This led to the optimized repulsion and attraction terms: C_{N-C} = 11 335 498.2 kcal Å^{12}/mol and D_{N-C} = 1 174 346.7 kcal Å^{10}/mol. Similar terms, part of the standard AMBER force field,[30] were included for the water−cation interactions, *viz.* C_{H-OW} = 7557.0 Å^{12}/mol and D_H−OW = 2385.0 Å^{10}/mol, in order to ensure an appropriate balance with the π-cation interactions. It should be clearly understood that, by including a short-range 10−12 potential function to describe the interactions between toluene and ammonium, we do not imply that we have reached an optimal representation of charge-dipole or charge-quadrupole interactions—as a genuine nonadditive force field would have—but rather that we have found an economical way to correct underestimated binding free energies *via* a simple two-body potential.[17,18] It should be also understood that, in doing so, water−cation or water−toluene interactions are not affected by the improvement of the toluene−ammonium pair potential—as they would have been if explicit polarizabilities, and, hence, "multibody" effects, were involved. The incorporation of short-range 10−12 terms for a correct description of π-cation interactions in molecular dynamics simulations is currently being tested on systems of biological interest, such as the binding of tetrahydroacrydine (THA)—a synthetic drug for the Alzheimer's disease—with acetylcholinesterase (AChE).[51]

For the PMF simulation in an aqueous medium, a parallelepipedic periodic box containing 527 TIP3P[52] water molecules was used to describe the solvent (*i.e.*, approximately 28.1 × 24.6 × 23.1 Å^3). The dielectric constant in eq 2 was set to 1.0.

All the PMF simulations were performed using a hard cutoff of 9.0 Å to truncate both the solute−solvent and solvent−solvent interactions. Duffy *et al.* have shown, employing similar conditions on related molecular systems, that increasing the intersolute separation (hence, the nonbonded cutoff) does not modify the PMF curve significantly.[53] To put the free energy profiles on an absolute scale, the free energy of interaction was set to zero at 8.0 Å. The time step for integrating the MD trajectories was set to 1.0 fs, and the average pressure was kept at 1 atm.[54] In the case of the simulation in TIP3P water, the average temperature was maintained at 300 K using the Berendsen algorithm,[54] with a separate coupling to an external heat bath for the solute and for the solvent.

All the chemical bonds were constrained to their equilibrium value by means of the SHAKE[55,56] procedure. In addition to the computations in TIP3P water, we have generated free energy profiles *in vacuo*, employing both the standard and the modified (*i.e.*, including an additional 10−12 term) models. All the PMF simulations presented in this paper were preceded by an appropriate equilibration of the molecular system.

3. Results: Discussion

1. Gas-Phase Simulations. The free energy profiles representing the approach of the ammonium ion toward the aromatic ring of toluene, in the gas phase—using the two previously described additive models—are shown in Figure 2. The deep minimum of these curves suggests a strong noncovalent interaction between the cation and the "anionic" π-system.

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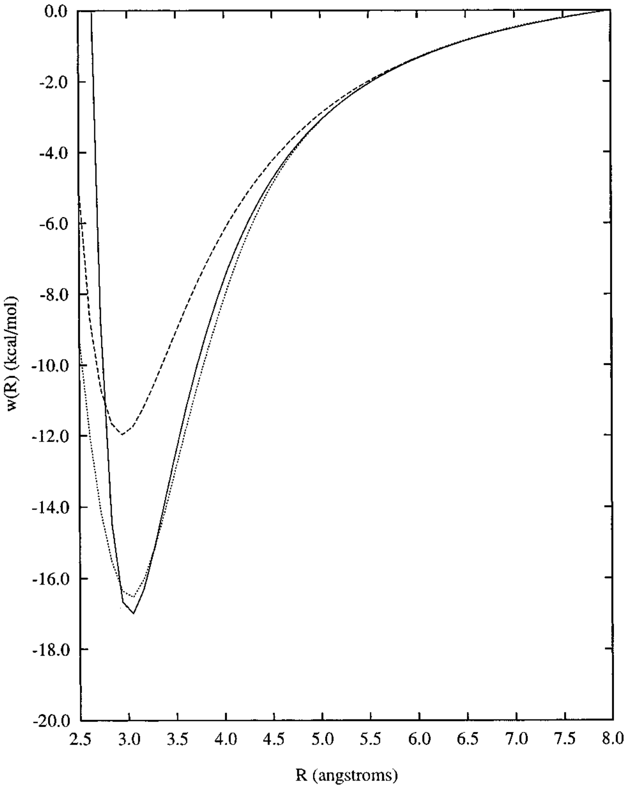

Figure 2. Toluene−ammonium free energy profiles in the gas phase: axially constrained along the C_6 symmetry axis of the aromatic ring, using (solid line) or not (dashed line) a short-range 10−12 potential between the nitrogen atom of ammonium and the carbon atoms of the aromatic ring; unconstrained and using a 10−12 potential (dotted line).

Table 2. Estimation Relative Free Energies of the "Contact" and the "Solvent-Separated" Complexes of Toluene and Ammonium, *in Vacuo* and in TIP3P Water

	"contact"		"solvent-separated"	
minimum	R_{N-D} (Å)	$w(R_{N-D})$ (kcal/mol)	R_{N-D} (Å)	$w(R_{N-D})$ (kcal/mol)
in vacuo				
constrained	3.05[a]	−16.99[a]		
	2.94	−11.97		
unconstrained	3.05[a]	−16.53[a]		
TIP3P water				
constrained	3.05[a]	−5.47[a]	5.69[a]	−0.45[a]
	3.16	−0.76	5.69	−1.02
unconstrained	3.16[a]	−2.99[a]		

[a] Inclusion of an additional 10−12 term between the nitrogen and the carbon atoms of the aromatic ring.

This minimum occurs at an intermolecular distance of 3.05 or 2.94 Å, with a corresponding free energy of −16.99 or −11.97 kcal/mol, respectively, depending on whether the specific N−C 10−12 potential was added, or not (see Table 2). Characterizing the directionality of the present π-cation interaction is not obvious: examination of several snapshots, extracted from the two trajectories and corresponding to a fixed equilibrium distance of 3.05 and 2.94 Å—*i.e.*, with and without the extra 10−12 term—reveals that the complex oscillates between a monodentate and a bidentate motif. It is worth noting, however, that upon classical minimization, we found the monodentate complex to be energetically more favorable, regardless of the model adopted. For these minimum energy structures, which correspond to an intermolecular distance of 2.99 and 2.93 Å, a binding energy of −18.96 and −13.74 kcal/mol was obtained respectively in the presence and in the absence of the 10−12 potential.

Table 3. Estimation Relative Binding Energies for the Potentially Three Most Stable 1:1 π-Complexes of Toluene with the Ammonium Cation

	ab initio[b]		molecular mechanics	
complex[a]	R_{N-D} (Å)	ΔE^{MP_2} (kcal/mol)	R_{N-D} (Å)	ϵE^{AMBER} (kcal/mol)
monodentate	3.14	−18.23	3.00[c]	−18.96[c]
			2.93	−13.74
bidentate	3.03	−19.42	2.99[c]	−18.53[c]
tridentate	3.05	−17.64	2.99[c]	−17.81[c]

[a] See Figure 3. [b] Non-BSSE MP2/6-31G**//HF/6-31G** ab initio calculations. [c] Inclusion of an additional 10−12 term between the nitrogen and the carbon atoms of the aromatic ring.

As has been highlighted above, our choice to coerce the approach of ammonium toward toluene along its 6-fold symmetry axis finds its justification in earlier experimental observations, suggesting that aromatic rings are perpendicular to the N^+ groups of basic amino acids.[4,5] It would be instructive, however, to estimate the magnitude of the changes induced in the gas-phase free energy profile, when the cation approaches the toluene centroid in an orientationally-averaged manner. To this end, we have repeated the preceding simulation, using the force field that includes the short-range 10−12 term, but removing the set of angular holonomic constraints $\{N, D, C_i\}$. As can be observed in Figure 2, the modifications entailed by the removal of these constraints—mostly related to entropic effects—are very minor. In particular, compared to the constrained profile, the depth of the minimum is raised by less than 0.5 kcal/mol (see Table 2).

In order to provide a rationalization of the π-cation interaction in the toluene−ammonium dimer, we have carried out a number of additional high-quality ab initio calculations. These computational studies of the three potentially most stable complexes (*viz.* the mono-, bi-, and tridentate motives) involve a complete geometry optimization at the Hartree−Fock level of approximation, using the split-valence 6-31G** basis set,[41] followed by a single point energy evaluation at the second-order Møller−Plesset[57] (MP2) level to take into account correlation effects. Closely related to the choice of an adequate basis set is the treatment of the basis set superposition error (BSSE). In the most general case, this is achieved by evaluating the counterpoise correction.[58] The frequent overestimation of the BSSE, however, has made this approach questionable, so that several authors have suggested increasing the basis set to the maximum affordable size rather than performing counterpoise correction calculations.[59,60] Since we are interested in relative—rather than absolute—binding energies, and assuming that the BSSE is approximately constant for the three configurations studied, these corrections will not be introduced. Moreover, Kearney *et al.*[11] have demonstrated that, on the related benzene−ammonium complex, the absolute BSSE correction was negligible. As may be seen in Table 3, the general trend of our ab initio calculations is in quantitative agreement with the recent theoretical studies of Kearney *et al.*[11] and Kim *et al.*,[1]17 and the pioneering work of Deakyne and Meot-Ner[3] on the benzene−ammonium dimer. In particular, the bidentate motif, in which two hydrogens point down toward the "anionic" face of the toluene ring, is found to be energetically preferred, with a corresponding relative binding energy of −19.42 kcal/mol (see Figure 3). This value is *ca.* 1.5 kcal/mol larger in magnitude than that observed with the ammonium−benzene complex, in similar conditions.

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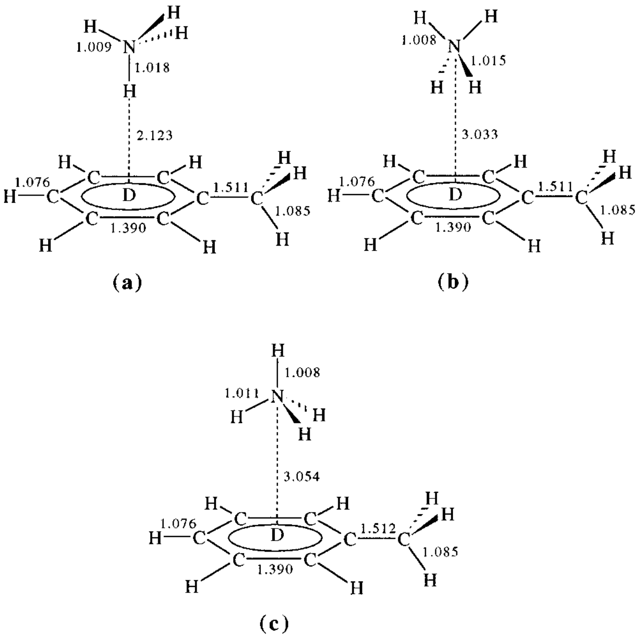

Figure 3. Ab initio HF/6-31G** optimized 1:1 complexes of toluene with ammonium: mono- (a), bi- (b), and tridentate (c) motives. All bond lengths in Å.

A comparison of the structures predicted to be most stable using molecular mechanics and ab initio geometry optimization reveals that, whereas the former predicts the monodentate motif to be most stable, the latter suggests the bidentate configuration is lowest in energy. However, the ab initio energy differences between the mono-, bi-, and tridentate structures are quite small.[61] The largest discrepancy between molecular and quantum mechanics lies in the absolute value of the binding energies, and, to a lesser extent, to the equilibrium intermolecular distances when the standard pure two-body additive model is used (see Tables 2 and 3).

So far, it would seem that the ab initio data described herein agree reasonably well with the molecular mechanics results obtained employing an artificial representation of polarization effects—at least, in the vicinity of the energy minimum, from which the short-range 10−12 potential between the nitrogen atom of ammonium and the carbon atoms of the aromatic ring (see Figure 1) was fitted. It is, however, not quite obvious whether the asymptotical behavior of the modified potential is totally satisfactory. In order to address this issue, we have plotted the potential energy difference ΔE^{AMBER} as a function of the distance separating the centroid of the toluene ring from the nitrogen atom of ammonium and compared the resulting profile (not represented here) with the ab initio energy differences ΔE^{MP_2} estimated from a series of MP2/6-31G**//HF/6-31G** single points. The deviation between the two sets of data is relatively minor, and tends to suggest that, at least in a vacuum, the molecular mechanics potential involving the extra 10−12 term offers an acceptable, albeit simplistic, representation of the toluene−ammonium π-cation interaction.

2. Toluene−Ammonium Association in Water. The PMFs depicted in Figure 4 represent the free energy profiles for bringing the two solutes toward each other in an aqueous solution, using the two aforementioned additive models. From the onset, it appears that this process in TIP3P water is thermodynamically favorable. While the PMF profiles obtained *in vacuo* have a single deep minimum located at 3.05 and 2.94 Å—whether the 10−12 term was included, or not—the curves generated in solution are not monotonic, and actually have two local minima at 3.05 and 5.69 Å, and at 3.16 and 5.69 Å, respectively with and without the N−C 10−12 potential (see Table 2). On account of the unquestionable qualitatively incorrect representation of π-cation interactions allowed by the standard two-body additive model, only the free energy minima obtained with the specific 10−12 term will be described in what follows:

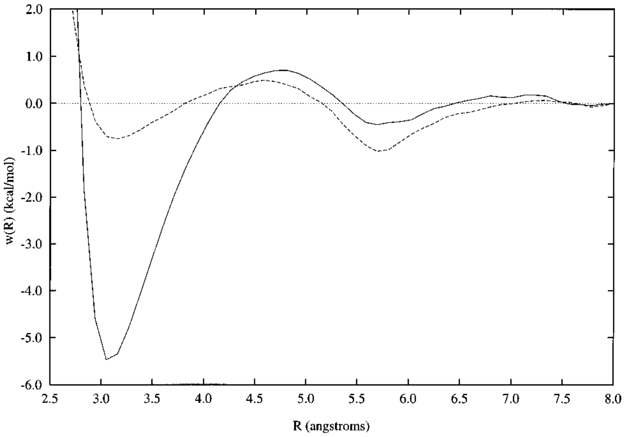

Figure 4. Toluene−ammonium potential of mean force in TIP3P[52] water, axially constrained along the C_6 symmetry axis of the aromatic ring, using (solid line) or not (dashed line) a short-range 10−12 potential between the nitrogen atom of ammonium and the carbon atoms of the toluene ring.

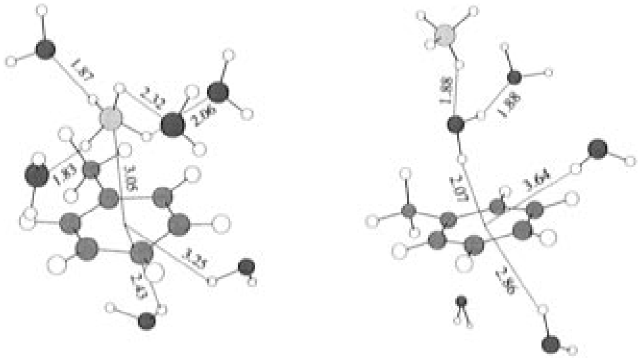

Figure 5. Random configuration of the hydrated toluene−ammonium dimer in the vicinity of the "contact distance" (a) and "solvent-separated" (b) minima. These snapshots were generated from individual 25 ps MD simulations, using the extra 10−12 potential.

(a) The first minimum (at 3.05 Å), referred to as the "contact" minimum, corresponds to an unconventional hydrogen bond (*viz.* 2.73 and 2.95 Å) between two of the ammonium hydrogens and the center of the aromatic ring (see Figure 5a). This local minimum occurs with a free energy of −5.47 kcal/mol—much lower than that estimated in the absence of the short-range 10−12 potential (*viz.* −0.76 kcal/mol). As may be observed in Figure 5a, the particular "close-contact" structure (*viz.* a quasibidentate motif) reflects a balance in sampling of the potentially two most stable dimers: the mono- and the bidentate complexes. This fact has been suggested previously by Duffy *et al.* for related π-cation interactions.[53] Interestingly, one may also note the presence, on the opposite face of toluene, of another hydrogen bond (*viz.* 2.43 Å) between one of the hydrogens of a water molecule and the center of the ring, hence further supporting that aromatic rings effectively behave as hydrogen bond acceptors.[62,63]

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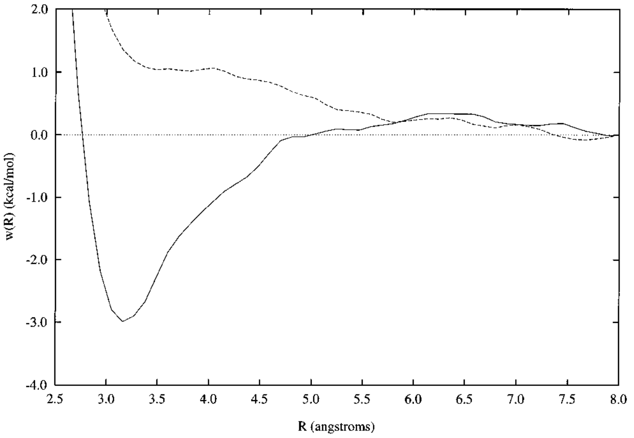

Figure 6. Toluene−ammonium potential of mean force in TIP3P[52] water, orientationally averaged, using (solid line) or not (dashed line) a short-range 10−12 potential between the nitrogen atom of ammonium and the carbon atoms of the toluene ring.

(b) The second minimum (5.69 Å), a "solvent-separated" minimum, should be considered more carefully. This minimum, with a corresponding free energy of −0.45 kcal/mol, is about 5 kcal/mol higher than the one arising at a "contact distance". The barrier separating the two minima is *ca.* 6.17 kcal/mol high, and occurs at 4.81 Å. A closer look at the configurations generated when ammonium and toluene are *ca.* 5.69 Å away from each other reveals that designating the minimum "solvent-separated" is certainly appropriate.[31] As may be seen in Figure 5b, there is a distinct water molecule located roughly half-way between the aromatic ring and the ammonium ion, forming hydrogen bonds to both solutes (*viz.* 2.07 and 1.88 Å, respectively).

Just like for the gas-phase free energy calculations, we have performed two additional PMF simulations in TIP3P water, removing the {N, D, C_i} angular holonomic constraints. The resulting PMF curve generated with the modified potential energy function *i.e.*, N−C 10−12 term), and depicted in Figure 6, reveals that only the "contact" minimum is stable, and that it occurs at 3.16 Å, with a free energy of *ca.* −2.99 kcal/mol (see Table 2). This observation is not really surprising, since the removal of the angular holonomic constraints contributes to increase the entropy of the system, hence reducing noticeably the depth of the "contact" free energy minimum. In contrast, when no specific 10−12 term is added to the standard potential, the derived free energy profile (see Figure 6) is conspicuously repulsive, albeit presenting traces of both the "contact" and the "solvent-separated" minima witnessed in the constrained PMF (see Figure 4).

Clearly, the above results suggest that toluene−ammonium association is far from negligible in TIP3P water. In addition, the free energy profile described herein unambiguously confirms that, when the onium group axially approaches toward the anionic face of the aromatic ring, a strong π-cation is observed at a "contact" distance, rather than at a "solvent-separated" one.[4,5] Considering both solvent effects and the entropic factors associated with thermal/configuration averaging, it is not totally surprising to witness such a considerable diminution of the well depths obtained *in vacuo*. We note, however, that the magnitude of the present π-cation interaction is consistent with the binding free energies in excess of −1 kcal/mol reported in the literature for similar interactions involving onium and guanidinium groups in the neighborhood of aromatic ligands or amino acid side chains.[8,7,64] In this respect, Duffy *et al.* have emphasized that, in such systems, concurrent interactions may occur, so that distinguishing the exact contribution of π-cation interactions from others, like π-stacking or ion pairing, may be difficult.[53] Nevertheless, an estimation of the association constant K_a between toluene and ammonium, obtained by integration[65,66] of the PMF to an adequate separation distance R_{cut}, such as

$$K_a = 4\pi \int_0^{R_{cut}} r^2 e^{-w(r)/RT}\, dr \qquad (3)$$

leads to $K_a = 6.53$ M^{-1} for the unconstrained free energy profile ($R_{cut} = 4.92$ Å) and 224.71 M^{-1} for the constrained one ($R_{cut} = 4.15$ Å), including the short-range 10−12 term in the potential energy function. The former is not out of line with the estimate of 2.7−3.3 M^{-1}, derived by Schneider *et al.*[64] from spectroscopic analysis of tetramethylammonium−benzene-like interactions. Besides, it should be noted, that, as has been reported by Kim *et al.*,[17] in the gas phase, the simply ammonium cation binds benzene stronger than tetramethylammonium (*viz.* approximately 7 kcal/mol), hence suggesting that the estimate provided by Schneider *et al.* should constitute a lower bound of the actual association constant for toluene and ammonium in water.

In an attempt to trace the origin of the reduced stability of the π-cation interaction in an aqueous solution—compared to what can be witnessed in a vacuum, we have derived the free energy profile (not represented here) corresponding to the approach of the ammonium ion toward water, in a gaseous state and under the conditions described above. The minimum for this complex, emerging at 2.67 Å with a free energy of −17.96 kcal/mol, is *ca.* 1 kcal/mol lower than that for the toluene−ammonium dimer, if use is made of the short-range 10−12 potential between nitrogen and the carbon atoms of the aromatic ring. Interestingly enough, standard minimization leads to an equilibrium distance separating ammonium from water equal to 2.77 Å, in excellent accord with the value of 2.772 Å estimated by Del Bene[67] at the MP4/6-31+G(2d,2p) level of approximation, and an energy difference of −22.46 kcal/mol, slightly lower than that recently reported by Kim *et al.*[17] (*viz.* −20.73 kcal/mol) and by Del Bene[67] (*viz.* −20.3 kcal/mol). From this, it may be inferred that water−ammonium and toluene−ammonium interactions are of comparable magnitude, and that—especially when the intersolute distance between the ion and the aromatic species is great—ammonium could interact preferentially with water, at the expense of the π-cation interaction.

3. Analysis of π-Cation Interactions in Proteins. The results presented herein support the view that the magnitude of the π-cation interaction is quite sensitive to its immediate environment. An examination of 404 nonredundant structures[68] of biological interest, provided by the Brookhaven Protein Data Bank[69,70] (PDB), supports the suggestion that the aromatic ring of Phe will form a π-interaction with the onium group of Lys. In addition, it appears that, among the 647 Phe−Lys interactions indexed, the great majority occur with the distance from the nitrogen to the center of the aromatic ring in the range of 4.5 to

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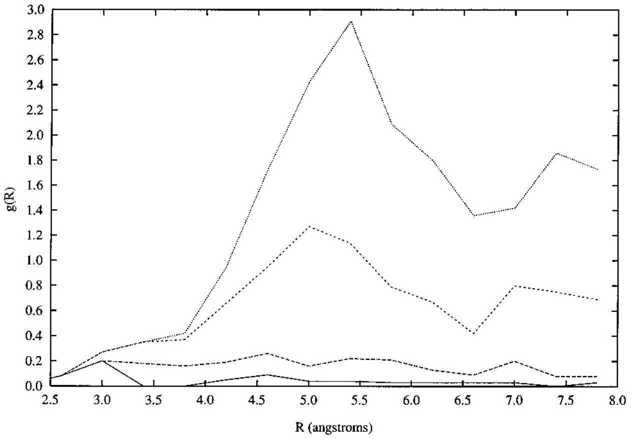

Figure 7. Nitrogen−aromatic ring centroid (N−D) distribution functions obtained from a statistical analysis of the Phe−Lys interactions in the Protein Data Bank.[69] Four different angles of approach of nitrogen toward the aromatic ring are considered (*i.e.* angle between the C_6 symmetry axis of the ring and the N−D bond): 10.0 (solid line), 20.0° (long dashed line), 40.0° (short dashed line), and 60.0° (dotted line).

6.0 Å, with a large angular scattering about the 6-fold symmetry axis of the aromatic ring (*viz.* 20.0−60.0°). In the few examples where the distance falls between *ca.* 3.1 and 4.0 Å, the approach of the positively charged functional group toward the center of the ring is almost perpendicular [see, for instance, the Phe^{174}−Lys^{259} interaction in the influenza virus hemagglutinin (1HGE)]. In Figure 7, we have plotted the normalized nitrogen−aromatic ring centroid distribution functions corresponding to three different angles of approach of the onium group toward the aromatic ring:

$$g_{\mathrm{N-D}} = \frac{\langle N_{\mathrm{D}}(\mathbf{r} + \delta\mathbf{r})\rangle}{\int 4\pi\partial_{\mathrm{D}} r^2 \, \mathrm{d}r} \simeq \frac{\langle N_{\mathrm{D}}(\mathbf{r} + \delta\mathbf{r})\rangle}{4\pi r^2 \delta r} \tag{4}$$

where $\langle N_{\mathrm{D}}(\mathbf{r} + \delta r)\rangle$ represents—for the chosen angle of interaction—the average number of Lys onium−Phe aromatic groups separated by a distance in the range $\mathbf{r}$ to $\mathbf{r} + \delta\mathbf{r}$ (distances measured between centroids), and ∂_{D} is the average density of Phe aromatic rings in the same sample set.

Whereas the studies of Burley and Petsko, and Singh and Thornton, considered the possible interactions between either Phe, Trp, or Tyr or Asn, Arg, Gln, or Lys, our statistical analysis focuses its attention on Phe−Lys interactions exclusively. We find that when the onium group approaches the center of the Phe ring within a 10.0°-aperture cone (*i.e.*, angle between the C_6 symmetry axis of the aromatic ring and the nitrogen−ring centroid bond), the maximum occurs slightly below 3.0 Å. Such contacts, characterizing "true" π-cation interactions, are still present when the aperture of the cone is increased to 20.0°, but an additional diffuse peak emerges around 4.5 Å. This secondary maximum of the normalized distribution function corresponds to concomitant interactions between the onium group, the "anionic" face of the aromatic ring, water molecules, and neighboring polar groups [see, for instance, the Phe^{204}−Lys^{209} interaction in the inorganic pyrophosphatase (1PYP)]. As may be seen in Figure 7, a further increase of the aperture reveals that the essential Phe−Lys interactions occur between 4.5 and 6.0 Å. While the first portion of the peak could actually correspond to pseudo-π-cation interactions, its maximum is not representative of π-cation interactions. In fact, around 6.0 Å, and beyond, we can consider that the side chains of Phe and Lys are not interacting anymore, but preferentially interact with neighboring functional groups. At such interatomic distances, even in the case of small angles of approach of nitrogen toward the ring centroid, there are a number of hydrophobic interactions between methylene groups of Lys and the aromatic ring of Phe [see, for instance, the Phe^{69}−Lys^{54} interaction in the glutaredoxin mutant (1ABA)]. This fact is in agreement with the recent work of Karlin *et al.*[71]

4. Conclusion

The simulations reported here demonstrate the role of the environment on π-cation interactions between two solutes. While aqueous solvation effects, together with thermal/configurational averaging, reduce the attractive well depth relative to the value observed *in vacuo*, the favorableness of association in water in clearly demonstrated.

The free energy calculations reported in this paper also ascertain that the magnitude and the directionality of π-cation interactions are closely related. Toward this end, we have derived two types of PMFs, incorporating, or not, a set of appropriate angular holonomic constraints to maintain the ammonium cation consistently perpendicular to the plane of the aromatic ring. While the axially constrained PMF shows in TIP3P water a deep "contact" minimum supplemented by a shallow "solvent-separated" one, the fully unconstrained free energy profile possesses only one "contact" minimum of reduced depth. It is our opinion that only the latter profile should be compared directly with experimental free energies of association, since, in essence, axial approach—as postulated from experimental observations[4]—is likely to occur at relatively moderate intersolute distances, rather than over the entire reaction path. Besides, it should be underlined that, in comparison with high-level ab initio calculations in the literature,[3,67,17] the magnitude of the water−ammonium interaction reported in this survey is overestimated by *ca.* 1.7 kcal/mol, whereas that of toluene−ammonium is underestimated by less than 0.5 kcal/mol. For this reason, the *ca.* 3.0 kcal/mol well depth of the unconstrained free energy profile could be viewed as a lower bound of the "real" value.

A comparison of our free energy profiles with the results of both Duffy *et al.*[53] and Gao *et al.*,[72] on the hydrated tetramethylammonium−benzene complex, using Monte Carlo and combined Quantum Mechanical/Molecular Mechanical (QM/MM) Monte Carlo simulations, respectively, shows a rough qualitative agreement. It is important to note that, although the π-cation interaction examined by these authors is different from the one analyzed here, their computed association constants in water turn out to be not too dissimilar from ours. The general shape of the PMF derived by Duffy *et al.*, however, is significantly different from that of Gao *et al.*—the first possessing a single strong "contact" minimum, and the second, a shallow "contact" minimum supplemented by a deep, broad "solvent-separated" one. In addition, their derived association constants of 33.8 and 0.76 M^{-1}, respectively, reflect a discrepancy likely to be rooted in their choice of energy function to reproduce the tetramethylammonium−benzene interaction. At this stage, it should be pointed out that the gas-phase molecular mechanics energy minima of −6.61 kcal/mol found by Duffy *et al.* and of −4.1 kcal/mol found by Gao *et al.* are respectively about 3 and 6 kcal/mol lower in magnitude than the value obtained from high-level ab initio calculations[17] and from experiment.[73] Despite this, the integration of their PMFs led to association constants (*viz.* 33.8 and 0.76 M^{-1}) that bracket our estimate of

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6.53 M^{-1} for toluene−ammonium. Of equal interest, the curve obtained by Duffy *et al*. closely resembles the one of our study that characterizes an orientationally-averaged mutual approach of the two solutes; in particular, the minima of the two PMF profiles are of comparable depth (*viz*. approximately 3.0 kcal/mol). Alternatively, the inclusion of a series of appropriate angular holonomic constraints—ensuring a perpendicular approach of the cation toward the aromatic ring—leads to a free energy profile that is somewhat similar to the one derived by Gao *et al*., who, incidently, also incorporated such types of constraints. Nevertheless, as has been noted,[53] the weak "contact" minimum found by Gao *et al*. in their PMF may be ascribed to the suboptimal reproduction of the tetramethylammonium−benzene gas-phase interaction energy by the AM1 method, which these authors employed in their QM/MM simulation. In all, although an approximate qualitative agreement can be found between our results and those of the aforementioned authors, we are still facing a lack of quantitative consensus; it is our hope that this paper will catalyze the determination of experimental quantities on simple π-cation complexes, such as the one presented here.

A problematic, yet unanswered question concerns the treatment of instantaneous polarization, especially in the case of molecular systems where polarization effects are envisioned to be far from negligible.[9,50,18] A closer look at the partitioning of the total interaction energy,[17] at the ab initio level of approximation, has provided a quantitation of the magnitude of the induction term in the toluene−ammonium complex. Such inductive effects can be incorporated in "classical mechanical" molecular models but, unfortunately, at considerably greater expense.[50,18] On the other hand, charge transfer effects can only be fully studied at quantum mechanical level. Nevertheless, although the use of a simple two-body additive model may not be completely adequate and rigorous to describe satisfactorily charge-dipole and charge-quadrupole interactions—and, in a more general sense, polarization effects—correct binding free energies between π-systems and cations may still be obtained, as has been reported in this study. The approach adopted for the present work, which consists of including a simple "short-range" attractive term in the potential energy function—although arguable, since it neglects "multibody" effects—is economical and provides quantitative agreement with both high-quality ab initio calculations and experimental data; but, more important, it ensures a balance between the strengths of water−cation and π-cation interactions. This balance is, in our view, the *sine qua non* condition for generating realistic PMF curves in aqueous solutions.

Finally, we note that, herein, we have presented novel applications of the TI/PF method[31] for calculating a PMF for nontrivial systems. The smooth appearance of the resulting free energy profiles confirms that PMF/TI constitutes a qualitatively correct and practical computational protocol for this kind of application.

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