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The Nature of Cation-π Binding: Interactions between Tetramethylammonium Ion and Benzene in Aqueous Solution

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ABSTRACT A combined quantum mechanical and molecular mechanical Monte Carlo simulation method was used to determine the free energy of binding between tetramethylammonium ion (TMA^+) and benzene in water. The computed free energy as a function of distance (the potential of mean force) has two minima that represent contact and solvent-separated complexes. These species are separated by a broad barrier of about 3 kJ/mol. The results are in good accord with experimental data and suggest that TMA^+ binds to benzene more favorably than to chloride ion, with an association constant of about 0.8 M^{-1}.

INTRODUCTION

Interactions between organic cations and protein aromatic residues are widespread and of considerable biological importance. It has long been recognized that protonated amines interact favorably with aromatic residues (1–4). More recently, analysis of the crystal structures of the quaternary amine-binding proteins acetylcholinesterase and immunoglobulin Fab McPC603 indicate that tryptophan and tyrosine residues constitute the first "solvation shell" in the binding sites for the quaternary ammonium moiety (5, 6). Mutagenesis studies of ion channel proteins suggest that aromatic sidechains are important determinants of the binding of quaternary amines to nicotinic acetylcholine receptors (7, 8) and to the putative pore region of Shaker K^+ channels (9, 10). In addition, synthetic host molecules that contain aromatic moieties have been found to bind quaternary amines with high affinity (11–13), and to catalyze reactions with cationic transition states through cation-π interactions (14).

In order to understand ligand-receptor binding equilibria it is necessary to quantify the free energies of bound and unbound species in water. Toward this end, we have investigated the cation-π interaction using a combined quantum mechanical and molecular mechanical (QM/MM) Monte Carlo simulation technique to compute the interaction free energy (the potential of mean force, or pmf) for a simple model of organic cation-aromatic binding: tetramethylammonium ion (TMA^+) and benzene in water. Some results have been presented in abstract form (15).

MATERIALS AND METHODS

In the combined quantum mechanical/molecular mechanical (QM/MM) approach (16–18) the solute molecules are described by the Hartree-Fock molecular orbital theory (19), while the solvent molecules are approximated by the three-site TIP3P model for water (20). The total effective Hamiltonian of the system is given by:

$$\hat{H}_{\text{eff}} = \hat{H}^{\circ} + \hat{H}_{\text{qm/mm}} + \hat{H}_{\text{mm}} \tag{1}$$

where $\hat{H}^{\circ}$ is the Hamiltonian for the QM molecules, $\hat{H}_{\text{mm}}$ is the solvent energy determined by the empirical TIP3P potential, and $\hat{H}_{\text{qm/mm}}$ is the solute-solvent interaction Hamiltonian defined previously (18, 21, 22). The solute-solvent interaction energy in the combined QM/MM force field is:

$$E_{\text{qm/mm}} = \langle \Phi | \hat{H}_{\text{qm/mm}} | \Phi \rangle + \sum_{i}^{\text{solute}} \sum_{s}^{\text{water}} 4\epsilon_{is}[(\sigma_{is}/R_{is})^{12} - (\sigma_{is}/R_{is})^{6}] \tag{2}$$

where Φ is the wave function of the solute molecules in aqueous solution, and the Lennard-Jones terms account for the dispersion interactions between the QM and MM regions that were omitted in the hybrid QM/MM approximation. The Lennard-Jones parameters are taken from our previous work (22). To form the QM/MM interaction Hamiltonian $\hat{H}_{\text{qm/mm}}$, a solute-solvent cutoff distance of 10 Å was used. Thus, any water molecules that are within the cutoff from either the ammonium nitrogen or the carbon atoms of benzene are included in the calculation. A cutoff distance of 9 Å between oxygen atoms of water was adopted for the solvent-solvent interactions.

Because the electronic degrees of freedom are explicitly included in the fluid simulations, a computationally efficient quantum mechanical method must be used. In the present study, the semiempirical Austin Model 1 (AM1) theory developed by Dewar and coworkers (23) was used to form the AM1/TIP3P force field. The AM1 method can yield excellent results for many organic systems, and is about 1000 times faster than ab initio calculations at comparable levels (18). Furthermore, the combined QM/MM Monte Carlo approach has the advantage over traditional methods that employ empirical potential functions (24–26), because the detailed electronic polarization of the aromatic π system from interactions with the cation and solvent molecules is explicitly treated. The reliability of the QM/MM method has recently been demonstrated in the evaluation of the solvent polarization effect and the estimation of solvation free energies for amino acids and nucleotide bases (22).

The pmf was computed via statistical perturbation theory (27) using the MCQUB/BOSS (28, 29), and MOPAC (30) programs. The simulations were carried out in a periodic rectangular box (20 × 20 × 30 Å) containing 390 water molecules plus benzene and TMA^+ at 25°C and 1 atm with the isothermal-isobaric (NPT) ensemble. The pmf was determined by computing free energy changes along the separation distance, R:

$$\Delta G(R1 \rightarrow R2) = -k_B T \ln \langle e^{-\Delta E(R1 \rightarrow R2)/k_B T} \rangle_1 \tag{3}$$

where k_B is Boltzmann's constant, $\langle\ \rangle_1$ represents the ensemble average using the Hamiltonian $\hat{H}(R_1)$, and $\Delta E(R_1 \rightarrow R_2)$ is the energy difference between solute separation distances $R1$ and $R2$. The perturbations were performed in the forward and backward directions (double-wide sampling) with ΔR equal to ± 0.1 Å. The separation of the two solute monomers was

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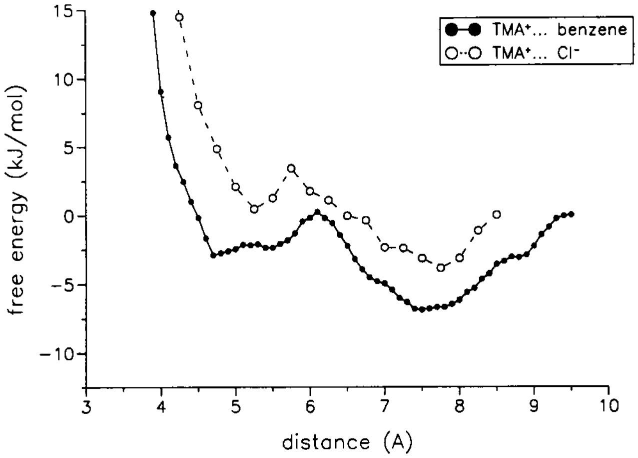

FIGURE 1 Computed potential of mean force for TMA$^+$. . .benzene (*solid curve*) and TMA$^+$. . .Cl$^-$ (*dashed curve* from Buckner and Jorgensen (32)) in aqueous solution at 25°C. The energy minima at 4.6 and 7.5 Å represent contact and solvent-separated pairs.

defined as the distance between the center of benzene and the nitrogen of tetramethylammonium ion. The computed pmf was anchored to zero at 9.5 Å because only relative free energies are available from the statistical perturbation calculations. Thus, the data shown in Fig. 1 came from 26 simulations, each consisting of at least 5×10^5 configurations for equilibration followed by 1.5×10^6 configurations for energy determination, and each requiring about 40 h to compute on an IBM RS/6000 Model 560 workstation. Standard errors were estimated by computing separate averages over blocks of 10^5 configurations. The cumulative standard deviation, computed from the middle of the pmf, was 0.8 kJ/mol.

The configurations were selected using Metropolis and preferential sampling, treating TMA$^+$ and benzene quantum-mechanically throughout. Full rotational degrees of freedom were sampled for TMA$^+$, but benzene was constrained to be perpendicular to the reaction coordinate. We imposed this constraint because tetraalkylammonium ion and aromatic residues form favorable face-centered complexes in proteins (1), and because the constrained reaction coordinate corresponds to the minimum energy path in the gas phase (3). Benzene should also be sampled angularly in the fluid simulations in order to more accurately compare the computed results to experimental K_a values.

RESULTS AND DISCUSSION

The computed pmf for TMA$^+$ and benzene in water is shown in Fig. 1. Two minima are apparent at separations of about 4.7 and 7.5 Å. These correspond to contact and solvent-separated pairs, respectively. Energetically, the computed well-depth of the contact minimum is 2.9 kJ/mol lower in energy relative to the species at a separation distance of 9.5 Å. The solvent-separated minimum in the pmf is broad and has a stronger interaction energy (7.5 kJ/mol) than the contact well. The barrier that separates the two species is also broad and peaks at about 6 Å with an activation free energy of about 3.1 kJ/mol for escaping from the cage of the contact pair.

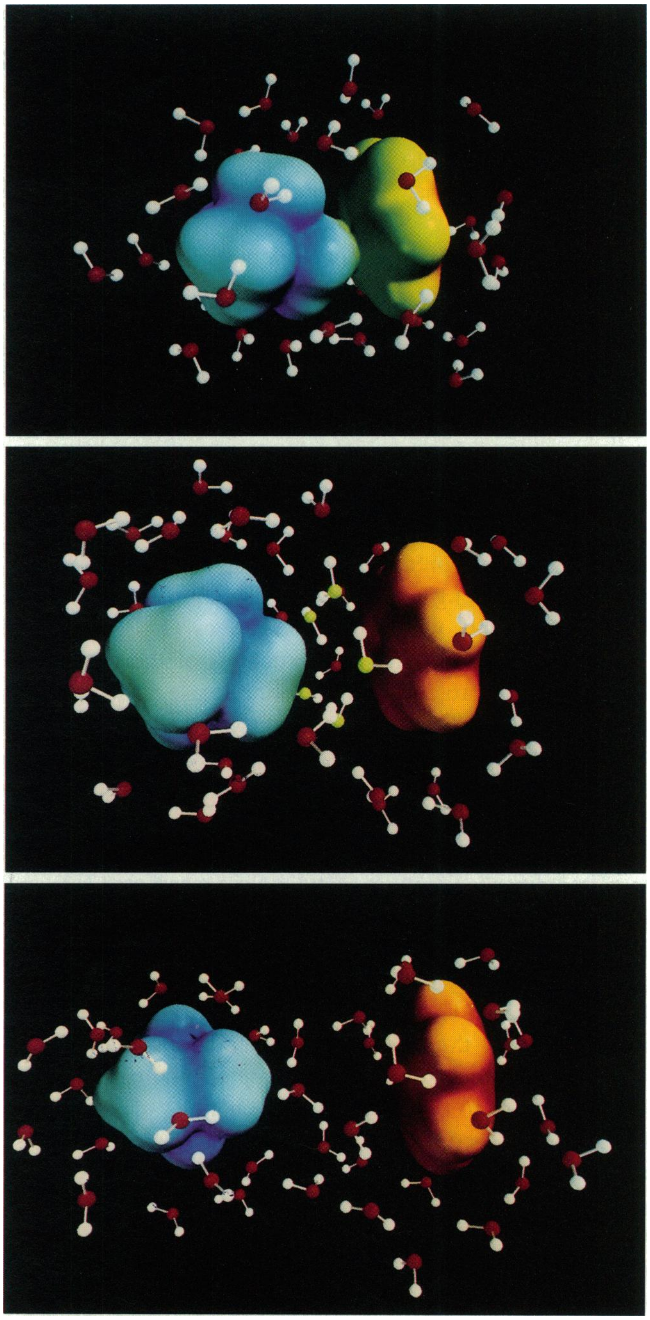

FIGURE 2 Plot of the final configurations from the Monte Carlo QM/MM simulations of tetramethylammonium ion and benzene in water at separations of 4.6 (*top*), 7.4 (*middle*), and 9.6 Å (*bottom*). For clarity, water molecules that are more than 4 Å from any solute atoms have been removed. The pentagonal water array between solutes at 7.4 Å is colored *green*, and the electron densities of the solutes are colored by the electrostatic potential (*blue* is positive and *red* is negative) to emphasize that solutes were treated quantum-mechanically, while the solvent, shown as a ball-and-stick model, was treated classically. This figure was made with the SPARTAN program (Wavefunction, Irvine, CA).

These computed energies are in accord with the experimental estimates of the binding free energy ΔG_b of 1.5–3 kJ/mol for cation-aryl interactions obtained from NMR and UV spectroscopic measurements of host-guest complexes in aqueous solution (31). For comparison, as part of the QM/MM simulation, the in vacuo enthalpy of interaction between benzene and TMA$^+$ was evaluated by AM1 geometry optimizations, and yielded a value of 17.2 kJ/mol. This, too, is in good agreement with the gas-phase binding free energy (14.6 kJ/mol) determined by high-pressure mass

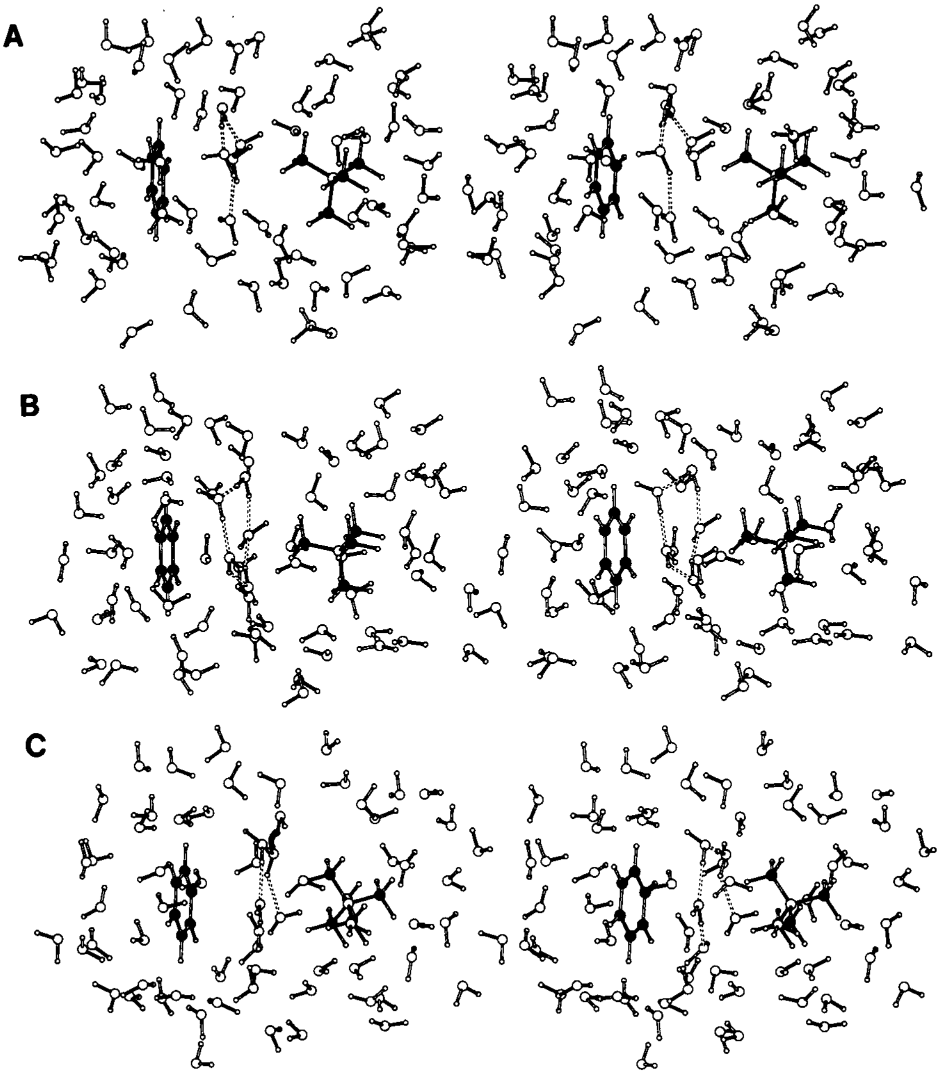

FIGURE 3 Stereoview of the structures after 5 × 10^5 (*middle*), 7 × 10^5 (*top*), and 10^6 (*bottom*) configurations during the simulation of TMA$^+$ and benzene pair in water at a separation of 7.4 Å. Water molecules more than 5 Å from any solute atom have been removed. Fluctuations of the pentagonal array of water molecules are clearly seen. The plot was made with the MindTool Program written by J. Blake and J. Tirado-Rives.

spectrometry (3). Thus, the simulation results suggest that aqueous solvation reduces tetramethylammonium ion-benzene binding by about 14 kJ/mol. The agreement between our theoretical results and the experimental data further supports the validity of the QM/MM computational procedure.

To illustrate the interaction with solvent molecules, the last configurations from the Monte Carlo simulations at 4.6, 7.4, and 9.4 Å are shown in Fig. 2. At the contact minimum there are no solvent molecules between the two solutes. In contrast, the plot of the solvent-separated pair shows a layer of five water molecules forming an approximately planar, cyclic hydrogen-bonding network that is roughly parallel to the benzene surface. At larger separations, additional water molecules interpose between the solutes, and the solvent along the reaction coordinate loses its characteristic structure.

To investigate the stability of the pentagonal water structure at the 7.4-Å separation, we examined every 100,000th configuration generated during the simulation, from the first structure after equilibration to the last computed configuration. The cyclic water structure appeared after the first 100,000 configurations and remained throughout the rest of the simulation, although fluctuations of the hydrogen-bond distances were apparent (Fig. 3 *b*). The time scale of the structural fluctuations can be further characterized using molecular dynamics simulations.

The pmf shown in Fig. 1 may be compared with that for the tetramethylammonium chloride ion pair obtained by Buckner and Jorgensen (32) (*dotted curve* in Fig. 1). It should be emphasized that empirical potential functions were employed in their investigation, whereas the combined QM/MM AM1/TIP3P model was adopted in our study. The combined QM/MM method has the advantage taking into account of the solvent polarization effect on the solute molecules, which is expected to be important for benzene's π system. The shapes of the pmfs for the two systems are similar, although the well depths for TMA$^+$. . .benzene are somewhat deeper than for TMA$^+$. . .Cl$^-$. In addition, the contact ion pair for TMA$^+$. . .Cl$^-$ is located at 5.25 Å, substantially greater than that predicted for TMA$^+$. . .benzene. Note that the TMA$^+$. . .Cl$^-$ curve was anchored to zero at 8.75 Å, with energies computed according to a continuum electrostatic model.

This result is consistent with the much better hydrogen-bonding interaction between chloride ion and water (55 kJ/mol) (33) than that between benzene and water (7.5 kJ/mol) (34). In the case of $TMA^+ \ldots Cl^-$, by keeping the ions farther apart, the chloride ion can retain nearly all the hydrogen bonds in the first solvation layer (32), while for $TMA^+ \ldots$benzene, the hydrophobic interaction between benzene and water favors a closer contact with the organic tetramethylammonium ion.

The association constant K_a between benzene and tetramethylammonium ion was obtained by integrating the pmf to an appropriate separation distance, R_c (Eq. 2) (35, 36):

$$K_a = 4\pi \int_0^{R_c} r^2 \exp[-\Delta G(r)/RT]\mathrm{d}r \qquad (4)$$

The calculated K_a is sensitive to R_c, but a natural choice would be at the peak of the barrier that divides the contact and solvent-separated complexes. Integrating the data shown in Fig. 1 to this point (6 Å) gives a K_a of 0.76 M^{-1}. This is less than the estimate of approximately 2 M^{-1} from the spectroscopically derived binding free energy ΔG_b (K_a = exp-[$-\Delta G_b/RT$]) (31). The experimental value is matched in our calculations by choosing $R_c = 7.0$ Å. It is interesting to compare the computed and experimental binding affinities between TMA^+ and benzene with those for the $TMA^+ \ldots Cl^-$ dimer, where association constants (in water) have been estimated to be 0.3 M^{-1} from theory (32), and 0.8 ± 0.2 M^{-1} from conductance data (37). Thus both theory and experiment suggest that tetramethylammonium ion binds about 2.5 times more strongly to benzene than to chloride ion in aqueous solution.

Finally, the computed K_a for tetramethylammonium ion and benzene in water may be compared with the experimental K_a values for TMA^+ binding to proteins and to synthetic host-guest systems. Two acetylcholine-binding proteins, acetylcholinesterase (38) and the nicotinic receptor channel (39), each bind TMA^+ with an apparent K_a of 100–1000 M^{-1}, i.e., with substantially higher affinity than that estimated for the contact complex in the model system. Even if we extend the integration of Eq. 4 to 9 Å to include the solvent-separated species, the computed K_a of the model system (10.5 M^{-1}) is still 10–100 times smaller than the experimental values. While it is difficult to decompose the overall observed association free energy in a protein into specific contributions, stabilization from multiple and different aromatic residues, and a hydrophobic binding site in the protein's interior, could certainly account for the discrepancy. With regard to cation-π interactions in synthetically designed host-guest systems, Petti et al. (13) report a free energy of binding of TMA^+ to a host molecule that has six aromatic rings of 13.8–18.8 kJ/mol. Although the interpretation of these values, as well as those of Schneider at al. (31), is complicated both by the possibility of ion pair stabilizations (14, 40), as well as by our constraint that benzene remain perpendicular to the reaction coordinate, the energies are nonetheless in rough accord with our theoretical results that indicate a 3 kJ/mol well for the interaction of TMA^+ with a single benzene in water.

In summary, our calculations of specific $TMA^+ \ldots$benzene interactions in water indicate that the organic cation-aromatic π interaction facilitates the formation of a contact pair that is perhaps more stable than the ion pair between tetraalkylammonium ions and anionic residues in aqueous solution. The accord of the calculated and experimentally obtained results indicate that the simple model system may be extended to include other aromatic systems, cations, and solvents.

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