

Theoretical Calculations of Heteroconjugation Equilibrium Constants in Systems Modeling Acid–Base Interactions in Side Chains of Biomolecules Using the Potential of Mean Force

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The potentials of mean force (PMFs) were determined for systems forming molecular and ionic heterocomplexes (in which the proton is transferred from a neutral acid to a neutral base molecule) composed of acetic acid and phenol with amines in three solvents with different polarities and hydrogen-bonding propensities: acetonitrile (AN), dimethyl sulfoxide (DMSO), and water. For each pair and each solvent, a series of umbrella-sampling molecular dynamics simulations with the AMBER force field and explicit solvent molecules were carried out and the PMF was calculated by using the weighted histogram analysis method (WHAM). Subsequently, heteroconjugation equilibrium constants were calculated by numerical integration of the respective PMF profiles. All complexes except those involving acetic acid as a proton donor in water were found to form in solution, which was manifested by the presence of contact minima corresponding to hydrogen-bonded species in the PMF curves. The calculated heteroconjugation constants were found to be greater for complexes with proton transfer than for those without proton transfer for the acetic acid–*n*-butylamine system in all solvents and for the phenol–imidazole and phenol–*n*-butylamine systems in DMSO when the same reference state (molecular acid and molecular base) was considered. For the acetic acid–*n*-butylamine system in acetonitrile and dimethyl sulfoxide, the calculated constants of ionic heterocomplex formation are greater than the values determined by potentiometric titration, while the calculated constants of molecular heterocomplex formation, as well as the calculated constants of heterocomplex formation from acetic acid and 4(5)-methylimidazole, are in good agreement with the experimental data, which suggests that the force field applied overestimates the energy of the interactions of oppositely charged ions or the energy of the interactions of hydrogen-bonding species with solvent molecules. The constants were found to decrease with an increasing polarity and hydrogen-bonding propensity of the solvent (i.e., in the series AN > DMSO > H₂O), this being in agreement with the available experimental data.

Introduction

Hydrogen-bonding interactions between organic acid and base molecules capable of proton exchange play important roles in biological systems.^{1–7} These interactions contribute to the stabilization of protein structures through the formation of salt bridges and other interactions between the polar groups of amino acid side chains.^{1–4} Hydrogen bonding and subsequent proton exchange is a key step in a substantial number of enzymatic reactions, such as those catalyzed by serine and cysteine proteases, metalloproteases, lysosyme, and the respiratory-chain enzymes.^{5–7} Because these phenomena occur inside a macromolecular environment, experimental studies of their energetics and dynamics are difficult. The molecular modeling methods are an appropriate tool for such studies; however, because empirical force fields need to be applied for practical reasons, the accuracy of the results is uncertain. Therefore, comparing the results of calculations of model systems in solvents with different polarities and hydrogen-bonding propensities (which can model different environments encountered inside a macromolecule) with the available experimental data enables one to assess the accuracy of molecular simulations.

A model of acid–base equilibria^{8,9} that likely takes place between organic acids and bases includes dissociation of a molecular acid (1) and the cationic acid conjugated to the base (2), formation of molecular heteroconjugated complexes (3), and ionic heteroconjugation (4); the equations referred to also define the respective equilibrium constants K_a , K_b , K_{hetn} , and K_{heti} :



where A[−] is the anionic base conjugated with the molecular acid HA, BH⁺ is the cationic acid conjugated with the organic base B, and ⋯ denotes a hydrogen bond.

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To date, only limited simulation studies of model hydrogen-bonded systems in aqueous and nonaqueous solvents were reported in the literature.^{10–12} Chipot et al.¹⁰ calculated the potential of mean force (PMF) of a pair of guanidinium and acetate ions in water. They found a contact minimum at 4.1 Å and a broad solvent-separated minimum at 6.4 Å. Gervasio et al.¹¹ calculated the PMFs of the tryptophan–histidine pair in water, dimethyl sulfoxide (DMSO), methanol, and carbon tetrachloride. The respective PMFs showed that the interacting ionic pair was more stable in nonpolar solvents than in water, this being in good agreement with protein structural data. A more extensive study of the PMFs of the models of pairs of oppositely- and like-charged as well as charged and uncharged amino acid side chains in water was carried out recently by Masunov and Lazaridis¹² with the use of the CHARMM force field and a spherical water cluster with solvent spherical boundary potential (SSBP). The orientation of the interacting side chains was constrained to “head-to-head” (this providing optimum hydrogen-bond geometry) or “side-to-side”. The strongest interactions were found to occur between the arginine cationic acid and glutamic acid anion oriented head-to-head.

In our earlier work,¹³ by using the potentiometric titration method, we determined the heteroconjugation constants of the acetic acid–*n*-butylamine system. We also carried out¹⁴ *ab initio* calculations of the energetics of the heterocomplex formation of systems modeling acid–base interactions between amino acid side chains in peptides and proteins. In these calculations, acetic acid and phenol were proton donors and the organic bases were proton acceptors. The variation of the calculated free energies of the heteroconjugation reactions studied with the system was found to be in qualitative agreement with the variation of the logarithms of the heteroconjugation constants determined in various solvents. Very recently,¹⁵ we carried out molecular dynamics simulations with the AMBER¹⁷ force field of homoconjugating systems (i.e., systems forming hydrogen-bonded complexes between an acid or base molecule and its conjugated base or acid, respectively) involving model organic acids and bases and their conjugated bases and acids. We determined the PMFs of these systems and, subsequently, calculated the homoconjugation constants. We found¹⁵ that the agreement between the calculated and experimental constants was only qualitative; the calculated values were greater by 1–2 orders of magnitude than the experimental ones.

In this work, as a continuation of our earlier studies, we carried out molecular dynamics calculations with the AMBER force field of the formation of molecular and proton-transfer heterocomplexes between selected organic acids and bases modeling amino acid side chains, namely, acetic acid (AcOH; modeling the C-terminal carboxylic group and those of the aspartic and glutamic acids), phenol (PhOH; modeling the phenolic group of tyrosine), imidazole (Imid) and 4(5)-methylimidazole (MeImid; modeling the side chain of histidine), isopropylamine (iso-Prop; modeling the N-terminal amino group), 1-methylguanidine (MeGua; modeling the guanidine group of arginine), and *n*-butylamine (*n*-But; modeling the lysine side chain). Because only noncovalent interactions are involved, these systems can be treated at the molecular mechanics level, unlike the theoretical prediction of pK_a values in proteins, which has to be carried out at the QM/MM level.¹⁶

Methods

Molecular dynamics simulations were carried out with the AMBER¹⁷ suite of programs, using the AMBER 5.0 force field.¹⁸ Each system was placed in a $30 \times 27 \times 24$ Å³ periodic

box containing explicit solvent molecules with an amount corresponding to the experimental solvent density at 298 K (the solvents being water, acetonitrile, or DMSO), and simulations were carried out at 298 K in the NVT scheme (constant number of particles, volume, and temperature). A 10 Å cutoff for all nonbonded interactions, including electrostatic interactions, was imposed. The simulation time was 500 ps, and the integration step was 0.001 ps. A total number of 500 000 configurations was generated for each system.

The charges on the atoms of the solute molecules needed for the AMBER 5.0 force field were determined by using a standard procedure,¹⁹ by fitting the point-charge electrostatic potential to the molecular electrostatic potential computed using the electronic wave function calculated at the restricted Hartree–Fock (RHF) level with the 6-31 G* basis set. The program GAMESS²⁰ was used to carry out the quantum-mechanical calculations, while the program RESP¹⁹ of the AMBER 5.0 package was used to compute the fitted charges. The charges and the AMBER atom types are shown in Figure 1.

The force field parameters of the solvents were taken from ref 21 for water (the TIP3P model), from ref 22 for dimethyl sulfoxide (DMSO), and from ref 23 for acetonitrile (AN), respectively.

To determine the potentials of mean force of the systems studied, we carried out umbrella-sampling molecular dynamics simulations and processed the results by using the weighted histogram analysis method (WHAM).^{24–26} Harmonic-restraint potentials were imposed on the distance between the oxygen atom and the nitrogen atom (or the distances between all oxygen and nitrogen atoms), as illustrated in Figure 2. A bin size of 0.1 Å was chosen. With this bin size, the systematic error in the PMF, caused by the fact that the probability distribution and not the PMF is averaged within a bin, is <0.005 kcal/mol (given an average slope in the contact-minimum region of the PMF curves of ~ 2 kcal/Å); thus, the statistical error, on the basis of the “ruggedness” of the flat parts of the PMF curves at long distances, can be estimated to be ~ 0.1 kcal/mol. Both the statistical error and the systematic error are smaller than the depth of the minima, and the height of the maxima in the PMF curves and, therefore, the results are of sufficient quality to draw reliable conclusions. The calculations of the PMF were carried out as described in our earlier paper.⁹

The heteroconjugation equilibrium constants K_{hetn} and K_{heti} were determined from eq 5, as described in our previous paper:¹⁵

$$K_{\text{hetn}}(K_{\text{heti}}) = \frac{\int_{r_0}^{r_1} \exp\left(-\frac{W(r)}{RT}\right) 4\pi r^2 dr}{\frac{1}{V} \int_{r_1}^{\infty} \exp\left(-\frac{W(r)}{RT}\right) 4\pi r^2 dr} \quad (5)$$

where r_0 is the shortest distance found in the simulations, r_1 is the distance at which the first maximum in the PMF plot occurs, $r = 9$ Å for all cases, $W(r)$ is the potential of mean force (kcal/mol) at a given value of r ; R is the gas constant [8.314 J/(mol·K)]; T is the absolute temperature (298 K), and V is the volume ($V = 4/3\pi r^3$). Integration was carried out numerically.

Results and Discussion

The PMFs for all systems and solvents studied are plotted as functions of the oxygen–nitrogen atom distance in Figures 3, 4, 5, and 6. The logarithms of the molecular and ionic heteroconjugation constant values (obtained from the PMF

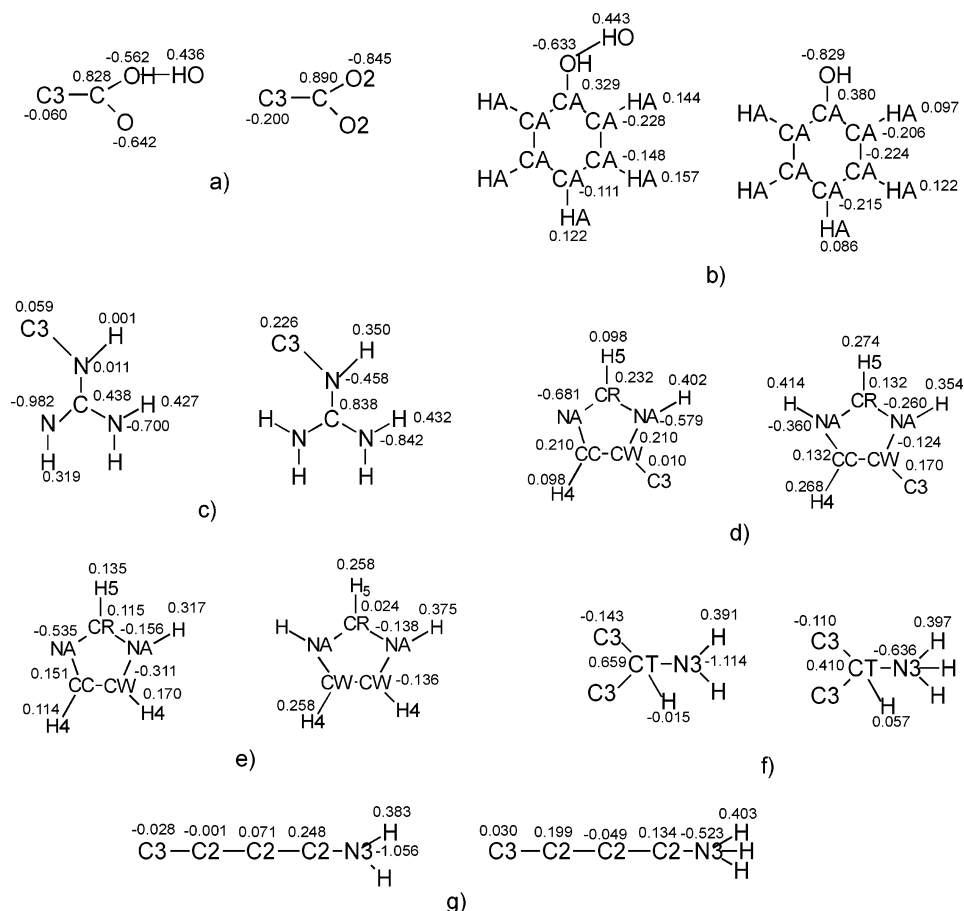


Figure 1. Partial atomic charges (in electron charge units) on the atoms of acetic acid and its anion (a), phenol and its anion (b), 1-methylguanidine and its cation (c), 4(5)-methylimidazole and its cation (d), imidazole and its cation (e), isopropylamine and its cation (f), *n*-butylamine and its cation (g).

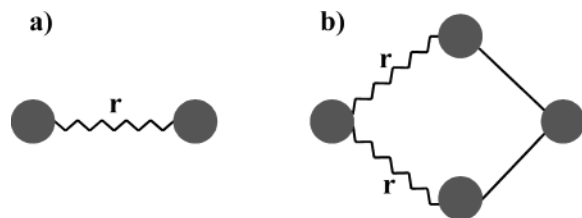


Figure 2. Illustration of the constraints imposed during molecular dynamics calculations. (a) A single restraint imposed on the amine nitrogen...carboxyl or phenolic oxygen distance, as implemented in the simulations of molecular heterocomplex systems and ionic heterocomplex systems, except for $\text{AcO}^- \cdots \text{amine cationic acid}$ and $\text{PhO}^- \cdots \text{MeGuaH}^+$. (b) Two restraints imposed on the carboxylate oxygen...amine nitrogen distance (for the $\text{AcO}^- \cdots \text{amine cationic acid}$ systems) or the phenolate oxygen... NH_2^+ nitrogen of MeGuaH^+ . For the $\text{AcO}^- \cdots \text{MeGuaH}^+$ system, one of the NH_2^+ nitrogen atoms was arbitrarily selected to define the restraints.

profiles following eq 5) for hydrogen-bonded systems are collected in Tables 1 and 2, respectively.

It can be seen (Table 1) that there is no tendency toward molecular heteroconjugation in aqueous solutions when acetic acid acts as the proton donor. In these systems (Figure 3, dotted line), the heteroconjugated species were not found to form, because there are no minima in the corresponding PMF profiles (Figure 3, dotted line). In contrast to this, in acetonitrile and dimethyl sulfoxide, the heteroconjugated species do form (there are contact minima in the PMF curves; Figure 3). Except for the case when imidazole acts as the proton acceptor, the heteroconjugation constant values are higher in acetonitrile than in DMSO (Table 1).

The distance dependences of the PMF for the acetic acid–amine systems are shown in Figure 3. A closer inspection of this figure reveals a similarity of the curves for acetonitrile to those for dimethyl sulfoxide, except for the acetic acid–imidazole system (Figure 3c). Contact minima that correspond to hydrogen-bonded complexes appear at 3.6–3.8 Å when acetic acid is the proton donor. Shallow solvent-separated minima between 5.2 and 6 Å can be observed in AN only.

The molecular heteroconjugation constants of the phenol–amine systems are by 1.5–3.5 orders of magnitude higher than those in the acetic acid systems (Table 1). This means that the molecular complexes of phenol and amines are much more stable than those formed by acetic acid. The most stable complexes are formed with 1-methylguanidine as the proton acceptor. The tendency toward the formation of molecular complexes in the phenol–amine systems is so strong that they appear even in water (where the logarithms of the formation constants range from 1.5 to 2.5), as opposed to the acetic acid–amine systems. It should also be noted that the molecular heteroconjugation constants decrease in the sequence $\text{AN} > \text{DMSO} > \text{H}_2\text{O}$, and the constants determined in acetonitrile are much greater than those determined in the remaining solvents, this agreeing with the increasing polarity and hydrogen-bonding propensity of the solvents in the series.

The PMF curves for molecular heterocomplex systems with phenol as the proton donor and organic amines as the proton acceptors for all the solvents studied are shown in Figure 4.

Contact minima corresponding to hydrogen-bonded complexes occur in the PMF curves (Figure 4) at a distance of ~2.6–2.7 Å for all solvents. The deepest minima occur for AN,

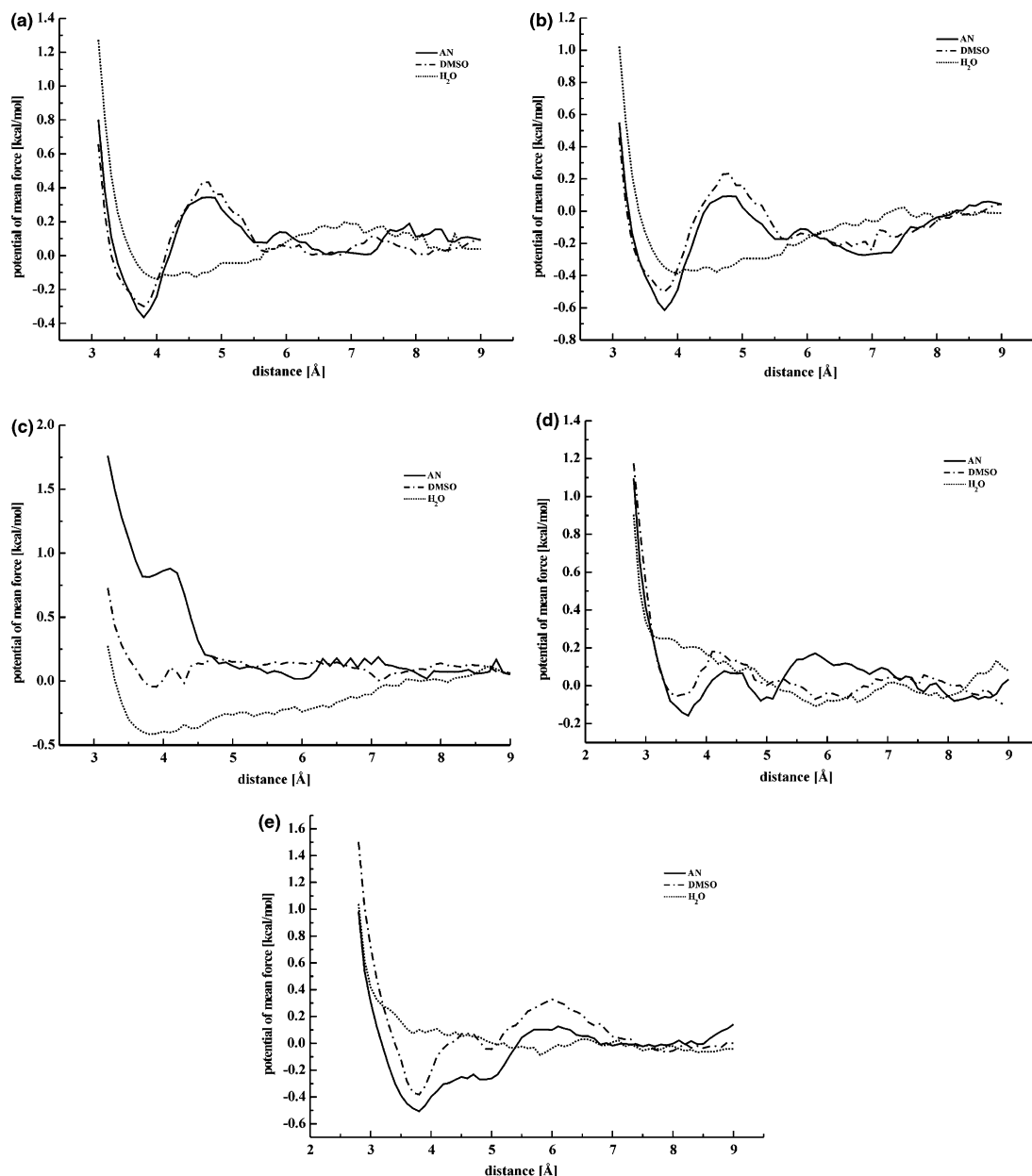


Figure 3. PMF profiles of the AcOH/MeGua (a), AcOH/MeImid (b), AcOH/Imid (c), AcOH/iso-Prop (d), and AcOH/*n*-But (e) in acetonitrile (solid lines), dimethyl sulfoxide (dot–dashed lines), and water (dotted lines).

TABLE 1: Logarithms of Molecular Heteroconjugation Constants, $\log K_{\text{hetn}}$, Determined by Molecular Dynamics Simulations with the AMBER Force Field and Explicit Solvent Models in Acetonitrile (AN), Dimethyl Sulfoxide (DMSO), and Water (H₂O)

system ^a	$\log K_{\text{hetn}}$			system ^a	$\log K_{\text{hetn}}$		
	AN	DMSO	H ₂ O		AN	DMSO	H ₂ O
AcOH/MeGua	2.86	2.17	<i>b</i>	PhOH/MeGua	6.02	4.56	1.52
AcOH/MeImid	2.65	2.58	<i>b</i>	PhOH/MeImid	5.25	1.16	2.33
AcOH/Imid	1.62	2.26	<i>b</i>	PhOH/Imid	2.72	2.32	1.34
AcOH/iso-Prop	2.36	2.31	<i>b</i>	PhOH/iso-Prop	4.76	2.75	2.53
AcOH/ <i>n</i> -But	2.83	2.61	<i>b</i>	PhOH/ <i>n</i> -But	4.43	3.15	2.57

^a Abbreviations for compounds: AcOH, acetic acid; PhOH, phenol; MeGua, 1-methylguanidine; MeImid, 4(5)-methylimidazole; Imid, imidazole; iso-Prop, isopropylamine; *n*-But, *n*-butylamine. ^b No minima in the corresponding PMF curve were found.

and the shallowest ones, for water, agreeing with the differentiating properties of the solvents. Shallow solvent-separated minima at a distance of 5–5.5 Å can be observed only for acetonitrile (Figure 4).

The logarithms of the ionic heteroconjugation constants (calculated from eq 5) are summarized in Table 2. A high tendency for salt-bridge formation in all the solvents studied (high $\log K'_{\text{hetn}}$ values) can be observed. The highest tendency

toward ionic heteroconjugation is observed in AN and DMSO for systems formed by $\text{AcO}^-/\text{iso-PropH}^+$ and $\text{AcO}^-/\text{n-ButH}^+$.

The PMF plots for all the ionic heteroconjugation systems studied in all the solvents are shown in Figures 5 and 6. The distance dependence of the PMFs of the acetate– and phenolate–organic ammonium ion systems is shown in Figures 5 and 6, respectively. In all curves, except for those of the systems with MeGuaH⁺ as the proton donor, deep and broad minima can be

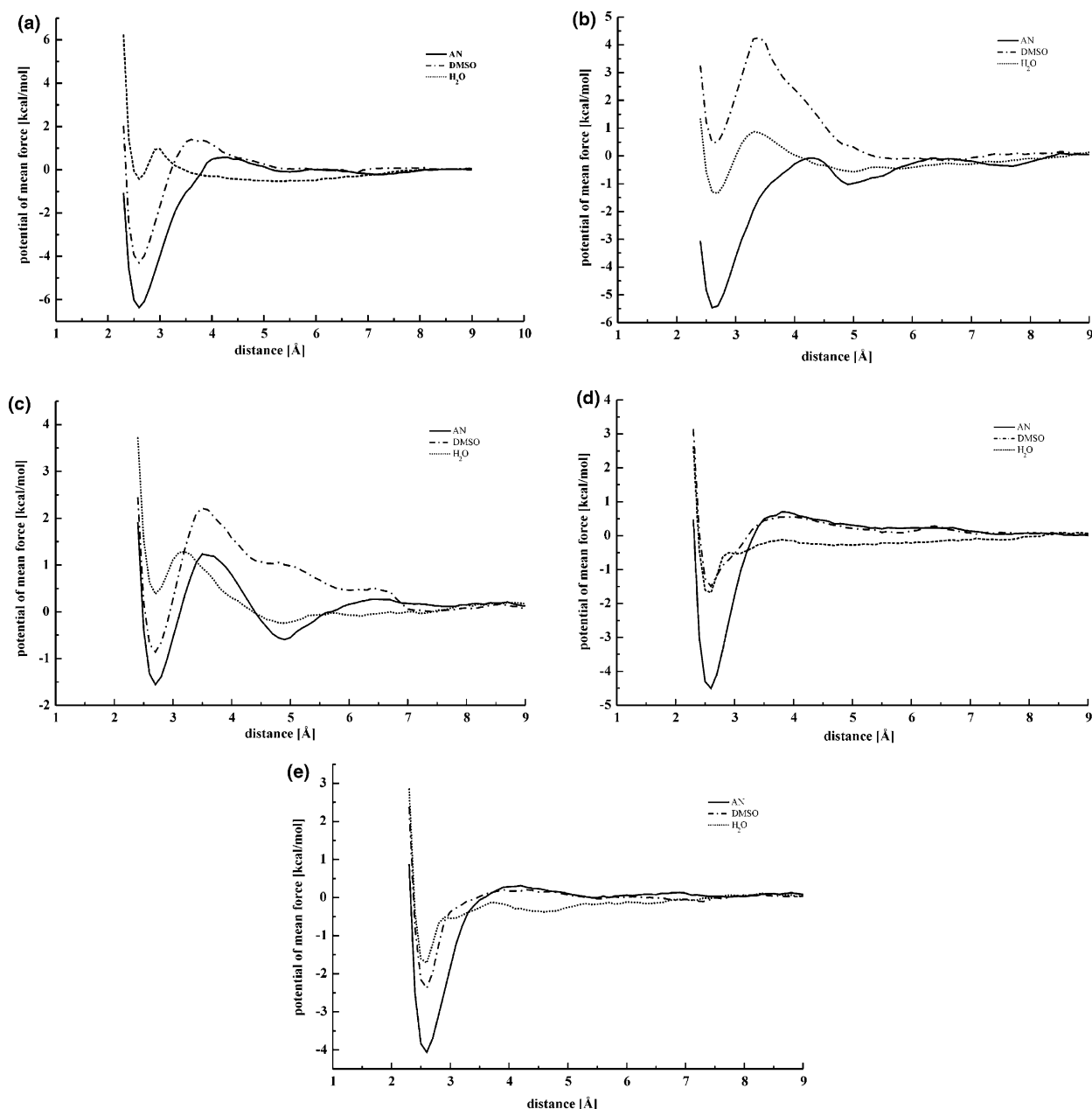


Figure 4. PMF profiles of the PhOH/MeGua (a), PhOH/MeImid (b), PhOH/Imid (c), PhOH/iso-Prop (d), and PhOH/*n*-But (e) in acetonitrile (solid lines), dimethyl sulfoxide (dot-dashed lines), and water (dotted lines).

TABLE 2: Logarithms of Ionic Heteroconjugation Constants, $\log K_{\text{heti}}$, Determined by Molecular Dynamics Calculations with the AMBER Force Field and Explicit Solvent Models in Acetonitrile (AN), Dimethyl Sulfoxide (DMSO), and Water (H₂O)

system ^a	$\log K_{\text{heti}}$			system ^a	$\log K_{\text{heti}}$		
	AN	DMSO	H ₂ O		AN	DMSO	H ₂ O
AcO ⁻ /MeGuaH ⁺	3.00	9.29	2.18	PhO ⁻ /MeGuaH ⁺	9.64	7.96	2.25
AcO ⁻ /MeImidH ⁺	8.96	8.80	1.63	PhO ⁻ /MeImidH ⁺	9.73	10.80	1.37
AcO ⁻ /ImidH ⁺	8.64	8.48	3.09	PhO ⁻ /ImidH ⁺	9.32	8.29	2.94
AcO ⁻ /iso-PropH ⁺	7.91	7.58	4.11	PhO ⁻ /iso-PropH ⁺	11.19	6.92	1.51
AcO ⁻ / <i>n</i> -ButH ⁺	7.04	6.83	4.28	PhO ⁻ / <i>n</i> -ButH ⁺	9.76	10.00	1.56

^a Abbreviations for compounds: AcO⁻, acetate anion; PhO⁻, phenolic anion; MeGuaH⁺, 1-methylguanidine cation; MeImidH⁺, 4(5)-methylimidazole cation; ImidH⁺, imidazole cation; iso-PropH⁺, isopropylamine cation; *n*-ButH⁺, *n*-butylamine cation.

observed at 2.6–2.7 Å. For the systems with MeGuaH⁺ as the proton acceptor, these minima appear close to 3 Å. For the acetate–*n*-butylamine cation systems, the depth of the contact minima in water is comparable with that determined by Masunov and Lazaridis¹² for the propionate–*n*-butylamine cation system (modeling the Glu⁻••Lys⁺ pair); these authors obtained a value of ~ -2.5 kcal/mol for the head-to-head

orientation and ~ -1.5 kcal/mol for the side-to-side orientation, respectively (Figure 4 in ref 12), while our value (averaged over all orientations) is ~ -1.88 kcal/mol (Figure 5e). Similarly, the depth of their minima of the propionate–methylimidazole cation system is ~ -1.7 kcal/mol (for the head-to-head orientation; Figure 5a in ref 12), while our value (averaged over all orientations) is -0.99 kcal/mol (Figure 5b). However, the depth

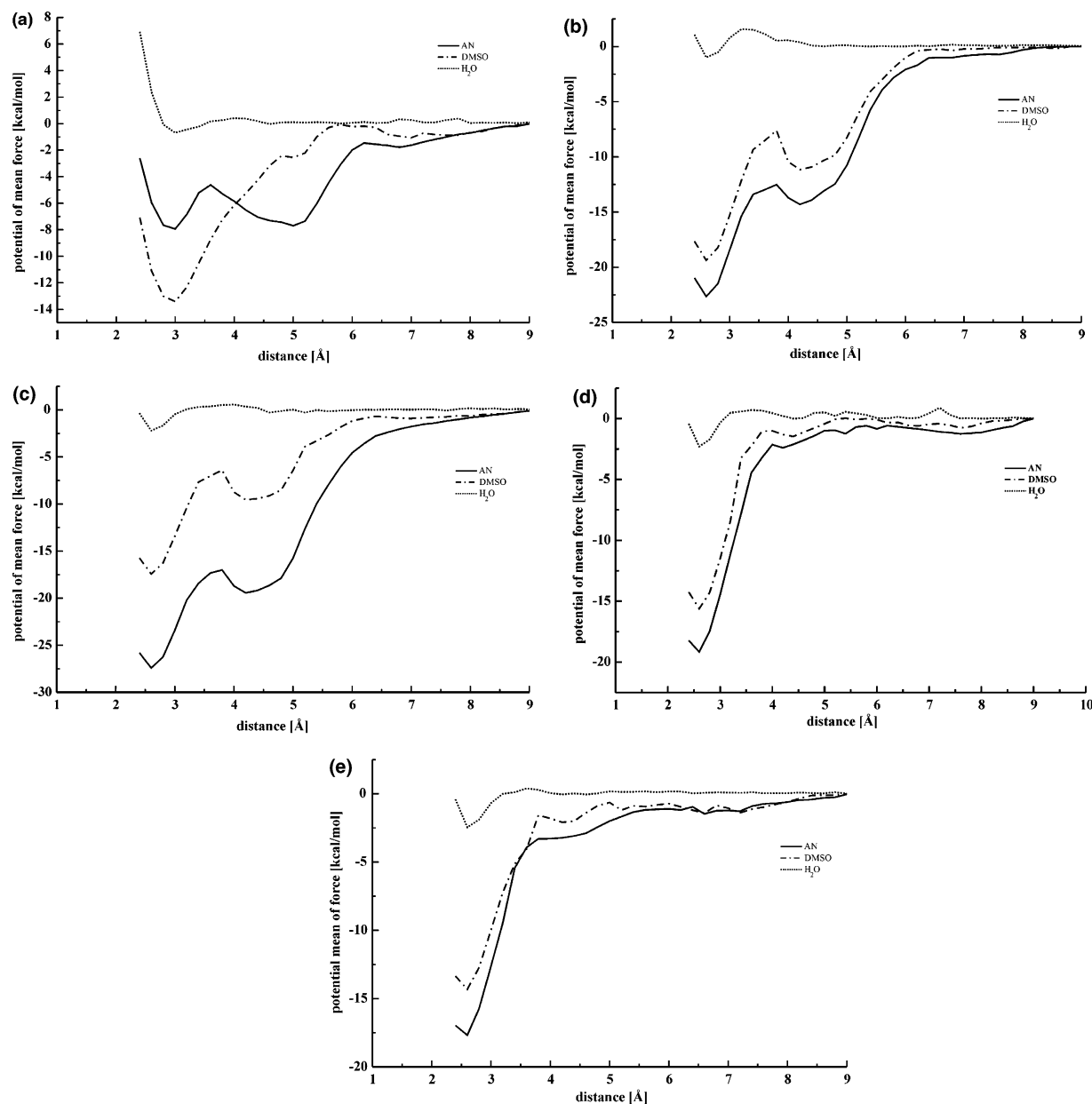


Figure 5. PMF profiles of the AcO[−]/MeGuaH⁺ (a), AcO[−]/MeImidH⁺ (b), AcO[−]/ImidH⁺ (c), AcO[−]/iso-PropH⁺ (d), and AcO[−]/n-ButH⁺ (e) in acetonitrile (solid lines), dimethyl sulfoxide (dot-dashed lines), and water (dotted lines).

of the minimum of the propionate–1-propylguanidine cation system calculated in their work for the head-to-head orientation is ~ -4.5 kcal/mol (Figure 3 in ref 12), while our value for the acetate–1-methylguanidinium cation system is only -0.53 kcal/mol. This discrepancy can be attributed to the fact that the orientation of the interacting species in their work was constrained, which resulted in the formation of two hydrogen bonds between the propionate oxygen atoms and the guanidine protons, while in our calculations the orientation was not restricted. It can also be observed that the PMF profiles calculated by Masunov and Lazaridis have a more developed structure with solvent-separated minima appearing for all systems, while the structure of the PMF curves obtained in our calculations is only residual (Figure 5). This difference can also be attributed to the fact that the orientation of the interacting species was restricted in the study of Masunov and Lazaridis and not restricted in ours.

To compare the ionic heteroconjugation constants with those of molecular heteroconjugation, a common reference system

must be used; we chose AH + B as the reference state. The redefined ionic heteroconjugation constant is defined by eq 6.

$$K'_{\text{heti}} = \frac{[\text{BH}^+ \cdots \text{A}^-]}{[\text{B}][\text{HA}]} \quad (6)$$

With this reference system, ionic heteroconjugation can be decomposed into two steps: (i) proton transfer from AH to B and (ii) formation of the $\text{BH}^+ \cdots \text{A}^-$ complex. Thus, the constant corresponding to the common reference state ($\log K'_{\text{heti}}$) can be calculated from that corresponding to the formation of the ionic heterocomplex from an ion pair from eq 7.

$$\log K'_{\text{heti}} = \log K_{\text{heti}} - \Delta pK_a \quad (7)$$

where ΔpK_a is the difference between the pK_a values of the proton acceptor and donor, while K_{heti} and K'_{heti} are defined by eqs 4 and 6, respectively.

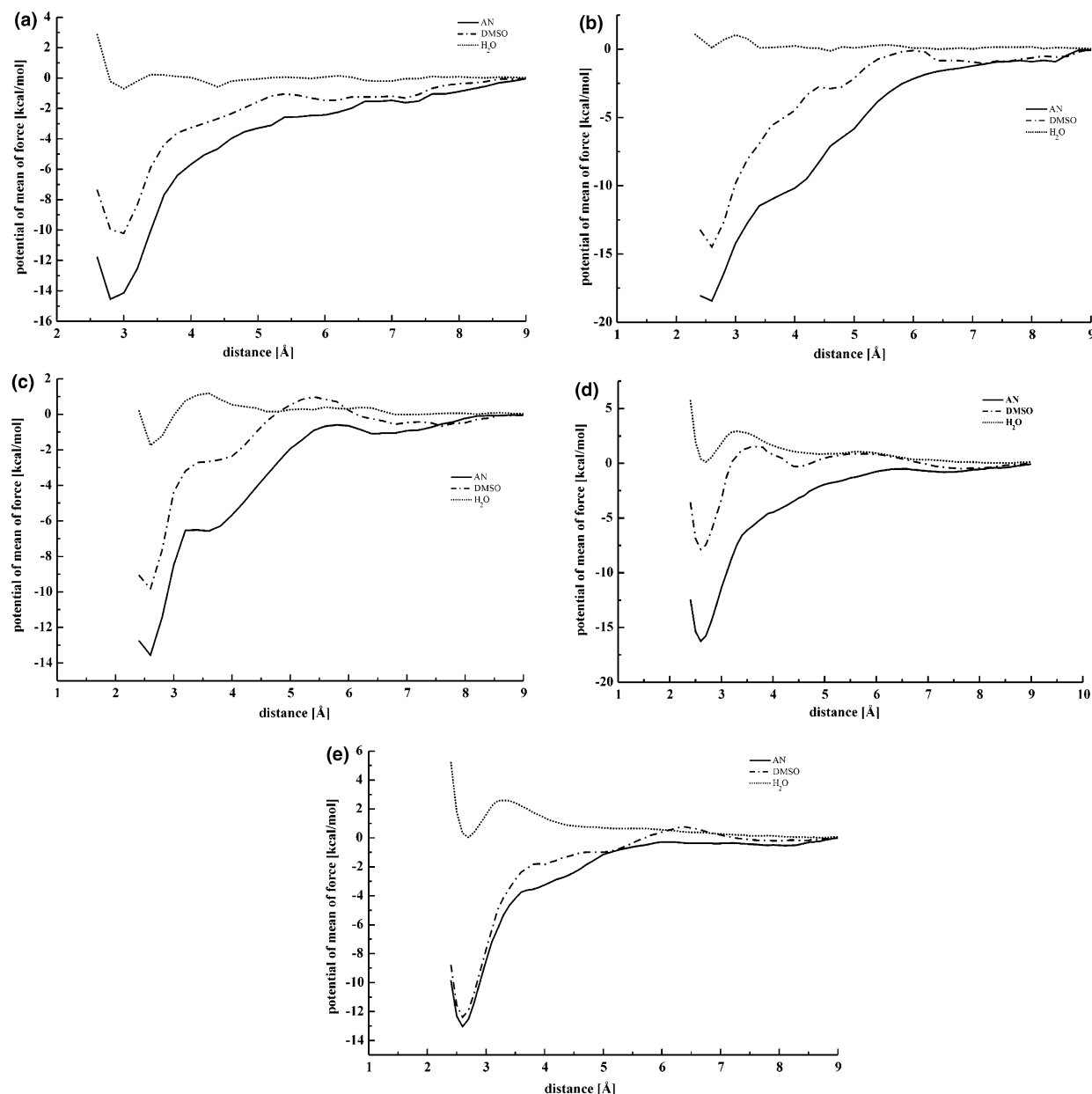


Figure 6. PMF profiles of the $\text{PhO}^-/\text{MeGuaH}^+$ (a), $\text{PhO}^-/\text{MeImidH}^+$ (b), $\text{PhO}^-/\text{ImidH}^+$ (c), $\text{PhO}^-/\text{iso-PropH}^+$ (d), and $\text{PhO}^-/\text{n-ButH}^+$ (e) in acetonitrile (solid lines), dimethyl sulfoxide (dot-dashed lines), and water (dotted lines).

The pK_a values available from the literature are collected in Table 3, and the values of $\log K'_{\text{heti}}$ calculated from the values of $\log K_{\text{heti}}$ from Table 2 and the pK_a values from Table 3 are summarized in Table 4.

As can be seen from Table 4, the ionic heteroconjugation constants calculated assuming the $\text{AH} + \text{B}$ reference state (K'_{heti}) are close to zero (their logarithms are negative numbers) or smaller than the values of the molecular heteroconjugation constants (K_{hetn}), except for the $\text{AcOH}/n\text{-But}$ system in acetonitrile and dimethyl sulfoxide and the PhOH/Imid and $\text{PhOH}/n\text{-But}$ systems in DMSO. On the basis of high values of K_{heti} for the $\text{AcOH}/\text{iso-Prop}$ and $\text{PhOH}/\text{iso-Prop}$ systems in acetonitrile and DMSO, the values of K_{hetn} for these systems can also be expected to be high; however, their pK_a values of isopropylamine in these solvents are unavailable. It is interesting to note that the values of K'_{heti} in water, which as a polar solvent could be expected to stabilize ionic heterocomplexes, are lower than the corresponding values of K_{hetn} ; therefore, molecular heterocomplexes can be expected to dominate in this solvent.

TABLE 3: Experimental pK_a Values of the Molecular Acids and Cationic Acids Conjugated to the Amines Studied in This Work at 298 K in Acetonitrile (AN), Dimethyl Sulfoxide (DMSO), and Water (H_2O)

system ^a	pK_a		
	AN	DMSO	H_2O
AcOH	21.56 ^b	11.75 ^b	4.75 ^c
PhOH	27.2 ^c	16.4 ^c	9.98 ^c
MeGua	<i>d</i>	28.5 ^e	13.4 ^f
MeImid	12.21 ^g	<i>d</i>	7.52 ^f
Imid	11.74 ^g	18.6 ^h	6.95 ^f
iso-Prop	<i>d</i>	<i>d</i>	10.63 ^f
<i>n</i> -But	18.26 ^b	9.70 ^b	10.66 ^f

^a Abbreviations for compounds: AcOH, acetic acid; PhOH, phenol; MeGua, 1-methylguanidine; MeImid, 4(5)-methylimidazole; Imid, imidazole; iso-Prop, isopropylamine; *n*-But, *n*-butylamine. ^b Reference 13. ^c Reference 28. ^d Experimental data not available. ^e Reference 29. ^f Reference 30. ^g Reference 27. ^h Reference 31.

The values of the heteroconjugation constants determined from potentiometric titration data are sums of K_{hetn} and K'_{heti} .

TABLE 4: Calculated Values of the Heteroconjugation Constants, $\log K'_{\text{heti}}$, in Acetonitrile (AN), Dimethyl Sulfoxide (DMSO), and Water (H_2O) Calculated from the Data of Tables 2 and 3 by Using Eq 7

system ^a	$\log K'_{\text{heti}}$		
	AN	DMSO	H_2O
$\text{AcO}^-/\text{MeGuaH}^+$	<i>b</i>	-7.46	-6.47
$\text{AcO}^-/\text{MeImidH}^+$	-0.39	<i>b</i>	-1.14
$\text{AcO}^-/\text{ImidH}^+$	-1.18	1.63	0.89
$\text{AcO}^-/\text{iso-PropH}^+$	<i>b</i>	<i>b</i>	-1.77
$\text{AcO}^-/n\text{-ButH}^+$	3.74	4.78	-1.63
$\text{PhO}^-/\text{MeGuaH}^+$	<i>b</i>	-4.14	-1.17
$\text{PhO}^-/\text{MeImidH}^+$	-5.27	<i>b</i>	-1.09
$\text{PhO}^-/\text{ImidH}^+$	-6.14	6.09	-0.09
$\text{PhO}^-/\text{iso-PropH}^+$	<i>b</i>	<i>b</i>	0.86
$\text{PhO}^-/n\text{-ButH}^+$	0.82	3.30	0.88

^a Abbreviations for compounds: AcO^- , acetate anion; PhO^- , phenolic anion; MeGuaH^+ , 1-methylguanidine cation; MeImidH^+ , 4(5)-methylimidazole cation; ImidH^+ , imidazole cation; iso-PropH^+ , isopropylamine cation; $n\text{-ButH}^+$, *n*-butylamine cation. ^b The value could not be calculated because the corresponding experimental $\text{p}K_{\text{a}}$ values are missing.

which can easily be realized from eq 8:

$$K_{\text{hetero}}^{\text{app}} = \frac{[\text{B} \cdots \text{HA}] + [\text{BH}^+ \cdots \text{A}^-]}{[\text{B}][\text{HA}]} = \frac{[\text{B} \cdots \text{HA}]}{[\text{B}][\text{HA}]} + \frac{[\text{BH}^+ \cdots \text{A}^-]}{[\text{B}][\text{HA}]} = K_{\text{hetn}} + K'_{\text{heti}} \quad (8)$$

where $K_{\text{hetero}}^{\text{app}}$ is the “apparent” heteroconjugation constant determinable from potentiometric measurements. Only limited experimental data on the systems studied in this work are available.^{13,27} For the acetic acid–*n*-butylamine system, the experimental values of $\log K_{\text{hetero}}^{\text{app}}$ are 2.25(0.19) and 1.37(0.79) in AN and DMSO, respectively (the numbers in parentheses are the standard deviations); these values are by an order of magnitude lower than the values of $K_{\text{hetero}}^{\text{app}}$ calculated from the values of Tables 1 and 4. The main contribution to $K_{\text{hetero}}^{\text{app}}$ comes from K'_{heti} , while the values of K_{hetn} (2.83 and 2.61, respectively) agree within the error margin (which can be taken as 3 times the standard deviation) with the experimental equilibrium constants. There also is good agreement between the heteroconjugation constants determined for the acetic acid–imidazole system in acetonitrile, the logarithm of the experimental heteroconjugation constant²⁷ being 1.95(0.10), compared to the calculated value of 1.62, which comes almost exclusively from K_{hetn} (Table 1) because the value K'_{heti} (Table 4) is negligible. It can, therefore, be concluded that the force field applied overestimates the interaction energy between charged hydrogen-bonded species or underestimates the interaction energy of charged species with the acetonitrile and dimethyl sulfoxide molecules. The second reason is more likely because the parametrization of these two solvents is not as advanced as that of water, for which a number of models have been published. Last, it should also be noted that the absence of polarization terms in the force field could also be the reason of imperfect agreement of the calculated constants with the experimental data.

Conclusions

Using umbrella-sampling molecular dynamics simulations, we determined the potentials of mean force corresponding to the formation of molecular and ionic hydrogen-bonded complexes between acetic acid and phenol and organic amines in

acetonitrile, dimethyl sulfoxide, and water. On the basis of the calculated PMF profiles, we calculated the heteroconjugation constants. For all systems, except for acetic acid and aliphatic amines in acetonitrile and dimethyl sulfoxide and imidazole and *n*-butylamine in DMSO, molecular heterocomplexes appear to be more stable than ionic heterocomplexes even in water, which could be expected to stabilize salt bridges. The values of the formation constants of ionic heterocomplexes in acetonitrile and DMSO appear too high, which suggests that the force field applied overestimates the energy of electrostatic interactions between charged systems or underestimates the energy of interactions of the solvent with charged hydrogen-bonding species. On the other hand, the values of molecular heteroconjugation constants are in good agreement with the limited experimental data that are available.^{13,27} The constants were also found to decrease with an increasing polarity and hydrogen-bonding propensity of the solvent (i.e., in the series $\text{AN} > \text{DMSO} > \text{H}_2\text{O}$), this being in agreement with the available experimental data.

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