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On the Interaction of Aliphatic Amines and Ammonium Ions with Carboxylic Acids in Solution and in Receptor Pockets

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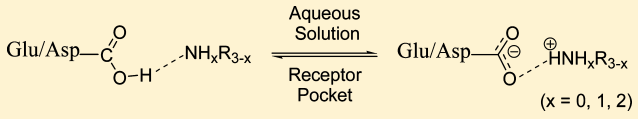
Supporting Information

ABSTRACT: Association energies of the acetate ion with cationic amines bearing one to three methyl groups were calculated in the range of −14 to −17 kcal/mol in aqueous solution by means of the IEF-PCM method at the CCSD(T)/CBS//MP2/aug-cc-pvdz and DFT/B97D/CBS//B97D/aug-cc-pvtz levels. The main stabilization factor for the association is the possibility for the formation of an ionic intermolecular hydrogen bond between the elements of the complex. For a quaternary ammonium ion, the favorable electrostatic interaction energy is the only driving force, and the stabilization energy for the complex is reduced to −4 kcal/mol. The internal free energies of the ion-pair tautomers of the studied species are higher by 10−15 kcal/mol in water than those for the neutral, hydrogen-bonded forms. Monte Carlo free energy perturbation calculations at T = 298 K and p = 1 atm predict −11 to −16 kcal/mol relative solvation free energy in favor of the corresponding ionic form. As a result, the ion-pair tautomer is the prevailing form in aqueous solution and on the extracellular surface of a receptor. Modeling the complex of a protonated ligand interacting with an Asp/Glu carboxylate side-chain in the binding cavity of a receptor, two strongly bound water molecules were considered so as to form hydrogen-bonded water bridges between the elements of the ion-pair. Nonetheless, the low polarity environment mimicked by a chloroform solvent cannot stabilize the ionic tautomer. A proton jump was predicted, which suggests that acetylcholine, an inherent cation by structure, might have evolved as the natural agonist for muscarinic receptors because a quaternary ammonium system assures the maintenance of the ion-pair form with a carboxylate side-chain in a protein cavity, the latter perhaps then being needed for receptor activation.



INTRODUCTION

It is generally appreciated that acetylcholine (Ach), the natural agonist for cholinergic receptors, is rather unique in its structural display of a permanently charged quaternary ammonium group (also referred to hereafter as a "quat"). In the most widely accepted models, Ach interacts with several flanking aromatic side-chains of the nicotinic receptor within a pocket formed by five helices, whereas at muscarinic receptors the principal interaction involves a discrete association with a distinct carboxylic acid side-chain.[1−4] At a physiological pH of 7.4, carboxylic acid groups are largely ionized. It is thought that formation of an ionic Ach−carboxylate interaction is an important step for activation of muscarinic receptors.

For either type of interaction, a fundamental question can be raised: Why does this natural agonist display a quat rather than one of the more prevalent primary, secondary, or tertiary amine systems which can likewise be expected to exist as cations due to protonation at physiological pH?[5] For example, several other neurotransmitters, including dopamine, histamine, serotonin, and norepinephrine, are primary amines, while epinephrine is a secondary amine. These compounds are agonists for different receptors, and all of them are thought to bind to a carboxylate side-chain in their corresponding receptor cavities.[3,4] As a group, these neurotransmitters can be considered to be 2-substituted ethylamines wherein their receptor specificities depend on distinct variations of the 2-substituent (Scheme 1). Ach can also be considered as a member of this family, although as already mentioned, the presence of its quat makes it unique. Primary, secondary, and tertiary amine molecules can form at least one ionic hydrogen bond to a carboxylate group. A priori, one can imagine that, because of steric effects, the C···N distance should be larger in a carboxylate···quat interaction compared to the amine systems where there can be 1−3 less sterically demanding N−H bonds present. Thus, follow-up questions relevant to muscarinic receptor interactions additionally arise. For instance, is the quat necessary to avoid $-COO^{-}\cdots{}^{+}H-N$ hydrogen-bond formation during the receptor activation process? Or alternatively, because ionic interaction is desirable, is the quat necessary to avoid a proton jump which would cause the ionic hydrogen bond to be replaced by a much weaker $-COOH\cdots NH_x$ (x = 0−2) interaction in the cases for the amine systems? To the best of our knowledge, these fundamental questions have not been studied at a high theoretical level.

When modeling ligand binding within a receptor cavity with explicit consideration of the nearest protein environment, consideration of a limited number of bound water molecules also may be needed. The latter, however, do not constitute an

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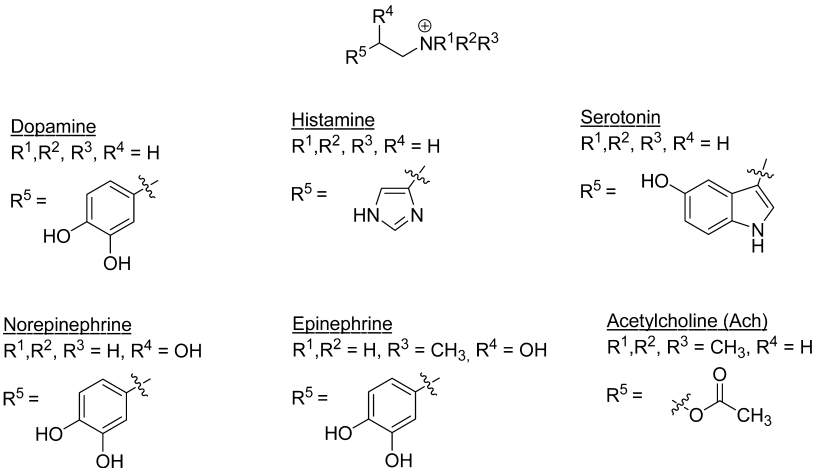
Scheme 1. Common Neurotransmitters Having a 2-Substituted Ethylamine Scaffold[a]



[a]Compounds are shown in their protonated states as would be expected at physiological pH. Also depicted is the presence of this scaffold within acetylcholine (Ach), which bears a permanently charged quaternary ammonium group rather than an amine.

aqueous solution environment. For such systems, QM/MM methods may be adequate, but computational problems could prevent the application of reliably high-level theoretical approaches for solutes much larger than water or methane.[6]

In a recent study,[7] the relative free energy for two kinds of Asp/Glu···Lys interactions was estimated within the depth of a protein. The latter environment was modeled by a polarizable continuum characterized by dielectric constants of ε = 5 and 15. It was found that the free energy of the complex is more favorable when the proton jumps over to the $-COO^-$ site, although the possibility for maintaining the ionic hydrogen-bonded system increases with increasing ε. The possible stabilizing effect of strongly bound water molecules was not considered in that study. The present investigation considers two water molecules forming stable hydrogen-bonded bridges between acetic acid and methylamine in their complex while adopting a chloroform environment (ε = 4.71) to model the nonaqueous nature of the receptor pocket. The arrangement with two bound water molecules is reasonable because the limited space in the binding cavity would keep the water molecules located near the largely polar receptor–ligand interface. An early paper[8] offers an explanation for the presence of water molecules at such interfaces, namely, that they can ameliorate the sometimes nonperfect steric and/or electrostatic fit between the partners. This could be even more important in the binding cavity of neurotransmitters, where at least two ligand–receptor connections may be necessary, and the effective length of the ligand in its favorable conformation may not meet the requirements in the absence of some water spacers.

Regarding carboxylate–amine interactions with surface receptors or enzymes, which are in contact with physiological fluid, aqueous solution models have been studied. One of these cases is the potential interaction of amines with sialyl glycopeptides,[9] a recent target of interest in our laboratory. The glycopeptides have different chains of sugar moieties forming an ether bond to the serine side-chains of the protein. An important structural feature of the sialyl sugars is a $-COOH$ group on their side-chain. This group is expected to be predominantly ionized such that it could favorably interact with protonated amines.

As indicated above, a proton jump from the protonated amine to the $-COO^-$ group is possible for forming the hydrogen-bonded neutral tautomer of the complex. Thus, in the present study, interaction energies of the acetate ion and protonated primary, secondary, and tertiary amines and the quaternary ammonium cation have been calculated. The relative free energies of the ionic complexes vs their neutral forms have been estimated in solution. The relative free energy for the tautomeric forms of the complex also has been estimated for the 2-methoxy-acetic acid and methylamine system so as to represent a model for the interactions with sialyl sugars.

A concern has emerged recently that continuum solvent models can both under- and overestimate the solvent effects through conformational/tautomeric transformations.[10,11] For comparison, Monte Carlo simulations considering explicit solvent molecules also have been performed herein for studying the possible proton jump in aqueous solution versus an environment characterized by a fairly low dielectric constant. Our ultimate goal is to quantitatively characterize the energy/free energy relationships for the ion association and the prediction of the prevailing form, ion-pair, or neutral hydrogen-bonded complex, in the binding cavity of a receptor and on a protein surface in contact with an aqueous solution.

METHODS AND CALCULATIONS

Continuum Solvent Calculations. As a first step of the theoretical study, the energy change through the process acid (aq) + amine (aq) = (acid···amine) (aq) was calculated. For the acid partner, acetic acid was selected, whereas the considered bases were methyl-, dimethyl-, trimethyl-, and tetramethyl (quat) amines. Since the partners are mainly ionized at pH 7.4 (see above), the deprotonated and protonated forms of the constituents of the complexes were considered, respectively. All complexes (with the exception of that formed with the tetramethylammonium cation) are, however, hydrogen-bonded species and may be considered as tautomeric systems depending on the position of the acidic proton. The thermodynamic probability of a proton jump was evaluated by comparison of the free energies for the ion-pair and the neutral form of the partners. In this respect, the 2-methoxy-acetic acid and methylamine pair was also studied. Henceforth,

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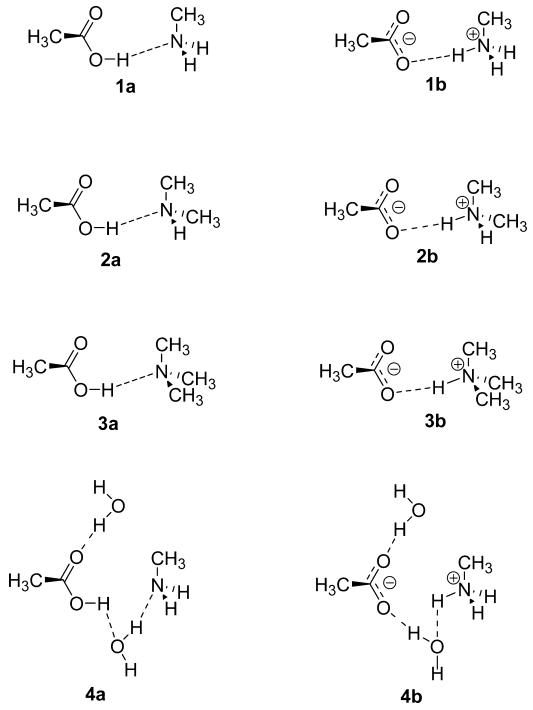
structures will be referred to as ion-pair or neutral complexes (with net zero charge for each) when the proton resides on the base or the acid partner, respectively. The orientations of the partners in the complexes are shown in Scheme 2.

Scheme 2. The Neutral (a) and the Ion-Pair (b) Forms of the Tautomeric Pairs for the Acetic Acid···Amine Complexes with Methylamine (1), Dimethylamine (2), and Trimethylamine (3)[a]



[a]The 4a and 4b pair shows the arrangement of two water molecules hydrogen-bonded to the acetic acid−methylamine complex. Only the ion-pair was considered for the acetate−tetramethyl ammonium (quaternary amine) complex.

Geometries of the complexes and those of the elements in their separated forms in aqueous solution were determined by using the IEF-PCM approach, the integral-equation formalism for the polarizable continuum method.[12−17] Applying scaled Bondi radii[18] for the cavity formation as described previously,[19] the geometry optimizations were performed at the MP2[20] level utilizing the aug-cc-pvdz basis set.[21−23]

The relative free energy for a tautomeric pair was calculated as

$$\Delta G_{tot} = (\Delta E^{s}_{int} + \Delta G_{th}) + (\Delta E_{elst} + \Delta G_{drc}) \equiv (\Delta E^{s}_{int} + \Delta G_{th}) + \Delta G(solv) \quad (1)$$

$$E^{s}_{int} = \langle \Psi | H | \Psi \rangle \quad (2a)$$

$$E_{elst} = \langle \Psi | {}^{1}/_{2} V | \Psi \rangle \quad (2b)$$

wherein H is the solute's Hamiltonian, V is the solvent reaction field generated by the fully polarized solute in solution, and Ψ is the converged wave function of the solute obtained from the in-solution calculation. E_{elst}, the electrostatic component of the free energy of solvation, is equal to G_{elst},[13,17] and ΔG_{drc} is the relative dispersion-repulsion-cavitation free energy. The dielectric constant of the water solvent was set to 78.39. The relative thermal correction, ΔG_{th}, was calculated as

$$\Delta G_{th} = \Delta ZPE + \Delta(H(T) - ZPE) - T\Delta S(T) \quad (3)$$

where ZPE is the zero-point energy and $H(T)$ and $S(T)$ are the enthalpy and the entropy, respectively, at T = 298 K and p = 1 atm, calculated in the rigid-rotor, harmonic oscillator approximation.[24]

The in-solution CCSD(T) (coupled cluster for singles and doubles with noniterative triples)[25,26] complete basis set relative internal energy was calculated using the formula by Hobza[27] as

$$\Delta E^{CCSD(T)}_{CBS} = \Delta E^{MP2}_{CBS} + (\Delta E^{CCSD(T)} - \Delta E^{MP2})_{aug\text{-}cc\text{-}pvdz} \quad (4)$$

ΔE^{MP2}_{CBS}, the complete basis set limit MP2 energy difference, was extrapolated by means of the formula[28−30]

$$E(CBS) = E(X) - A/X^3 \quad (5)$$

For calculating A and then E(CBS) in eq 5, aug-cc-pvtz single-point calculations were performed at the aug-cc-pvdz optimized geometries, and X was set to 2 and 3 with reference to the aug-cc-pvdz and aug-cc-pvtz energies, respectively.

The geometries for the hydrogen-bonded complexes were reoptimized at the DFT/B97D/aug-cc-pvtz level in aqueous solution. DFT/B97D[31] applies a direct correction for the intermolecular dispersion, which is not properly considered in several DFT methods. The CBS value for the B97D structure was obtained by utilizing eq 5. The IEF-PCM calculations were performed by means of the Gaussian 03 and 09 packages.[32]

For complexes with acetic acid, no precise local energy minima were reached except for the neutral acetic acid···dimethylamine and acetic acid···trimethylamine structures even after very long IEF-PCM/MP2/aug-cc-pvdz minimizations. As found in the previous study for the ion-pairs in solution,[7] relatively small imaginary frequencies in the range of 22i−45i were obtained, related to acetyl−methyl torsions. Using the default convergence criteria, the optimizations stopped when the predicted energy changes were in the order of 10^{-7}−10^{-6} au. The main intermolecular bond lengths were stable up to about 0.001 Å when the MP2/aug-cc-pvdz as well as the B97D/aug-cc-pvtz energies varied on the 10^{-6} au scale. The lowest energy structures for the complexes were accepted for further consideration. The problem of estimating ΔZPE and the relative thermal corrections in the absence of the lowest vibrational frequency is discussed in the next section.

Monte Carlo Simulations. Monte Carlo (MC) simulations were performed in NpT (isobaric−isothermal) ensembles at p = 1 atm and T = 298 K,[33−36] considering aqueous and chloroform solvent models. The applied methodology has been described in a number of recent papers,[10,11,37,38] and only the main technical points will be summarized here.

ΔG(solv), following the proton transfer of the carboxylic proton from the neutral acetic acid to a neutral amine in aqueous solution, was calculated by means of the free energy perturbation method (FEP)[39,40] as implemented in the BOSS 4.8 package.[41] The starting and final geometries of the complexes were determined previously by IEF-PCM geometry optimizations in the continuum dielectric solvent with ε = 78.39. For calculating the relative solvation free energy through the tautomeric process, one molecule of the complex was implemented in a box of 504 TIP4P water molecules.[42] The

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model corresponds to an infinitely dilute solution, in accord with the underlying assumptions in the PCM calculations.

In the applied FEP calculations, the geometry and simulation parameters of the neutral and ion-pair complexes were transformed into each other by means of a linear coupling parameter, λ. Since the net dipole moments change by 4−5 D (see the Supporting Information), forward and backward simulations were carried out with $\Delta\lambda = 0.025$ in order to test whether the perturbation conditions are satisfied.

Infinitely dilute solutions were simply modeled by applying a cutoff of 12 Å both for the solute−solvent and the solvent−solvent interactions. Each solute atom corresponded to a center for considering solute−solvent interactions if the central solvent atom was distanced by less than the cutoff radius (ICUT = 2).[41] In a reaction-field approach,[43] care was taken for the long-range electrostatic interactions beyond the cutoff limit and ε was set to 78.39. In this case, the explicit solvent molecules were considered within a sphere of R = 12 Å centered on the midpoint of the carboxylic carbon and the amine nitrogen atoms (ICUT = 0).

For a finite, 0.11 M solution model, all solvent molecules within a sphere of 12 Å around any solute atom were considered (ICUT = 2) and the long-range electrostatic correction was taken into account by Ewald summation[44,45] as implemented in BOSS 4.8. One molecule of the hydrogen-bonded complex was immersed in a solution box with an edge of 24−25 Å, corresponding to 9−10 dm^3 on the macro scale. The salt effect, present in biological systems, was considered by adding a dissolved Na^+/Cl^- ion-pair to the model, resulting in a composition near that in a 0.15 M isotonic saline solution.

For calculating the interatomic interactions, the all-atom OPLS-AA 12-6-1 pair-potential[46,47] was applied and the 12−6 Lennard-Jones parameters were taken from the BOSS 4.8 program's library. Considering the IEF-PCM/MP2/aug-cc-pvdz optimized geometries, the net atomic charges were obtained from their CHELPG fit[48] to the in-solution, IEF-PCM/B3LYP/aug-cc-pvtz molecular electrostatic potential, ELPO (Tables S1 and S2, Supporting Information).[49,50] This ELPO takes into consideration the charge transfer within the complex, and was generated by means of a wave function, which reflects the electron correlation for the solute and was developed on a large basis set. Using the aug-cc-pvtz basis set in MP2 single-point calculations, charges differed only by up to 0.035 atomic units (Table S1, Supporting Information). In B97D/MC simulations, the accepted geometries were obtained from IEF-PCM/B97D/aug-cc-pvtz optimizations and the charges were fitted to the corresponding ELPOs. The calculated dipoles varied in the ranges of 4.1−4.4 and 8.2−8.6 D for the neutral and ion-pair complexes, respectively, and differed by 0.1−0.6 D from those by the B3LYP parametrization. Since ΔG(solv) is a state function and depends only on the difference of G(solv) for the two end-points through the FEP process, the B97D/ΔG(solv) values were obtained from the corresponding MP2/ΔG(solv) values by transforming the two end-structures to the corresponding B97D structures by slightly changing geometries and atomic charges.

Calculations of MP2/ΔG(solv) for the $CH_3COOH\cdots H_2NCH_3$ to $CH_3COO^-\cdots {}^+H_3NCH_3$ tautomeric transformation were carried out in chloroform, using a single solute complex in a box of 124 chloroform molecules. The solvent box generated with the four-point chloroform model was taken from the library of BOSS 4.8. MC simulations without and with a reaction field were performed using cutoff radii of 12 Å, and ε was set to 4.71 in the reaction field approach. The solute charges obtained by the CHELPG fit to the IEF-PCM/B3LYP/aug-cc-pvtz ELPO are provided in Table S1, Supporting Information.

For modeling the proton transfer in a receptor cavity surrounded by a low dielectric environment, the $CH_3COOH\cdots 2H_2O\cdots H_2NCH_3$ to $CH_3COO^-\cdots 2H_2O\cdots {}^+H_3NCH_3$ transformation was followed in a box of 124 chloroform molecules. The reaction field radius was set to 12 Å, centered on the midpoint of the carboxylic carbon and the amine nitrogen atoms, and the dielectric constant was set to ε = 4.71. The water molecules were accepted to remain strongly bound to the complex in a low polarity environment, as suggested by the stability of the water dimer[51] and by the close contact for the protonated amine···chloride ion-pair[52] in dichloromethane. The ELPO fitted CHELPG charges for the solute atoms are provided in Table S3, Supporting Information.

RESULTS AND DISCUSSION

Geometries in Aqueous Solution. Table 1 shows the optimized intermolecular geometric parameters for the two different forms of the complexes. On the basis of MP2/aug-cc-pvdz optimizations, the C···N distances are in the range 3.15−3.23 Å when a strong hydrogen bond is comprised in the ion-pairs of the methyl-, dimethyl-, and trimethylamine with acetic acid. In the case of the quat partner, which can form only a weak C−H···O^- hydrogen bond to the carboxylate group, the C···N distance increases to 4.22 Å, presumably due to the quat's increased steric demand that is placed upon the system. Thus, the tetramethyl amine modeling of the Ach ligand in a receptor cavity is distanced by more than 1 Å from the carboxylate carbon atom compared with amines having only one to three methyl substituents.

The hydrogen-bond distances, X···H (X = O, N), decrease from 1.52 to 1.50 Å for the ionic acetic acid−amine complexes with increasing number of methyl groups on the nitrogen atoms. The decrease is much larger, from 1.57 to 1.45 Å, for the neutral complexes in the same series. The X···H distance of 1.56 Å shortens to 1.48 Å when the ionic form turns into the neutral one for the complex of 2-methoxy-acetic acid and methylamine. The O···H···N hydrogen bonds are slightly bent for any complex in Table 1, with bond angles varying between 169 and 178°.

IEF-PCM/B97D/aug-cc-pvtz geometry optimizations in water lead to C···N separations of 3.25−3.37 Å, and X···H (X = O, N) distances of 1.53−1.54 and 1.43−1.48 Å for the neutral and ion-pair complexes, respectively. The O···H···N hydrogen bond angle varies in the 176−179° range. All of these parameters describe complexes with C−N separations larger by 0.04−0.10 Å than that in the corresponding MP2-optimized structure. The X···H distances could be both shorter and longer by up to 0.09 Å, and the O···H···N moiety is almost linear.

For comparison, Table 1 provides MP2-optimized intermolecular geometry parameters for gas-phase complexes and for those in low-dielectric-constant environments. Stable complexes were obtained only for the neutral, hydrogen-bonded pairs in the gas phase. The results indicate a remarkable solvent effect on the parameters without clear-cut trends. For example, the (O)H···N hydrogen bond distance and the O···N distance are always considerably larger in the gas phase than in aqueous solution, but the C···N distance could be larger or smaller in the gas phase compared with that in the aqueous

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Table 1. MP2/aug-cc-pvdz and B97D/aug-cc-pvtz Optimized Intermolecular Geometric Parameters in Solution (IEF-PCM) and Gas Phase[a]

	C···N[b]	O···N[c]	O/N···H[c]	O···H···N	torsion
			$CH_3COOH\cdots NH_2CH_3$		
MP2 ε = 78.39	3.33	2.62	1.57[e]	176	
ε = 15[d]	3.33	2.63	1.58	176	
ε = 5[d]	3.33	2.65	1.61	176	
gas	3.23	2.70	1.69	169	
B97D ε = 78.39	3.37	2.61	1.54	176	
			$CH_3COO^-\cdots {}^+H_3NCH_3$		
MP2 ε = 78.39	3.15	2.61	1.52	171	
ε = 15[d]	3.12	2.59	1.49	171	
ε = 5[d]	3.06	2.54	1.40	171	
B97D ε = 78.39	3.25	2.57	1.43	176	
			$CH_3COO^-\cdots 2H_2O\cdots {}^+H_3NCH_3$		
MP2 ε = 78.39	4.30	3.64			
$O_{wat1}\cdots {}^+H_1(N)$			1.73	165	
$O_{wat2}\cdots {}^+H_2(N)$			1.78	156	
$H_{wat1}\cdots {}^-O_1$			1.65	177	
$H_{wat2}\cdots {}^-O_2$			1.66	169	
			$CH_3COO^-\cdots 2H_2O\cdots {}^+H_3NCH_3$		
MP2 ε = 4.71	4.23	3.59			
$O_{wat1}\cdots {}^+H_1(N)$			1.68	165	
$O_{wat2}\cdots {}^+H_2(N)$			1.73	158	
$H_{wat1}\cdots {}^-O_1$			1.60	176	
$H_{wat2}\cdots {}^-O_2$			1.62	170	
			$CH_3COOH\cdots NH(CH_3)_2$		
MP2 ε = 78.39	3.31	2.58	1.51[e]	175	
gas	3.22	2.67	1.65	171	
B97D ε = 78.39	3.36	2.61	1.53	176	
			$CH_3COO^-\cdots {}^+H_2N(CH_3)_2$		
MP2 ε = 78.39	3.16	2.61	1.51	174	
B97D ε = 78.39	3.26	2.59	1.46	176	
			$CH_3COOH\cdots N(CH_3)_3$		
MP2 ε = 78.39	3.27	2.55	1.45[e]	177	
gas	3.32	2.65	1.62	180	
B97D ε = 78.39	3.36	2.61	1.53	176	
			$CH_3COO^-\cdots {}^+HN(CH_3)_3$		
MP2 ε = 78.39	3.23	2.60	1.50	178	
B97D ε = 78.39	3.28	2.60	1.48	179	
			$CH_3COO^-\cdots {}^+N(CH_3)_4$		
MP2 ε = 78.39	4.22	3.64	2.13		
			$CH_3OCH_2COOH\cdots H_2NCH_3$		
MP2 ε = 78.39	3.26	2.56	1.48[e]	176	
OCCO(H)					20[e]
COCC					72
			$CH_3OCH_2COO^-\cdots {}^+H_3NCH_3$		
MP2 ε = 78.39	3.13	2.63	1.56	169	
$OCCO^-$					16[e]
COCC					72

[a]Distances in Å, angles in degrees. [b]C···N is the distance of the carboxylic carbon and the amine nitrogen atoms. [c]The shorter O···N distance, O···H(N) in the ion-pair, (O)H···N in the neutral pair. [d]C···N distance using geometries from ref 7. [e]Hydroxy oxygen in the neutral acetic acid and the corresponding O^- in the ion-pair.

solution. The increasing dielectric constants, ε = 5, 15, and 78.39, show for the studied acetic acid–methylamine complexes that the corresponding atom separations monotonically increase (with one exception) or decrease in the series.

A special acetic acid–methylamine complex was considered in the continuum dielectric model when two explicit water molecules were added to the system (4a and 4b in Scheme 2). The acidic side-chain in a protein is most likely ionized when it points toward bulk water, and a common aliphatic amine is protonated in aqueous solution at pH 7.4. The goal of the optimization of the $CH_3COO^-\cdots 2\ H_2O\cdots {}^+H_3NCH_3$ species in an aqueous environment was to explore whether the two water molecules form bridges and four hydrogen bonds between the elements of the ionic acid–base complex or the waters are repelled to the outskirt of the complex such that direct cation–anion interactions emerge. The calculated existence of four hydrogen bonds (Table 1) suggests that the water-bridged structure is at least a local energy minimum on the potential energy surface, at the expense of a much more separated C···N distance (4.30 vs 3.15 Å).

Complexes with Acetic Acid in Aqueous Solution. *Formation of Ion-Pairs.* Calculation of the relative standard free energy for the gas-phase protonation of isopropyl amine and guanidine[10] resulted in very good agreement with the experimental values upon the estimation of the complete basis set limit value for the potential energy change while applying the ideal-gas approximation and thermal corrections in the rigid-rotator, harmonic-oscillator approach.[24] The success in this case was partially attributed to the applicability of the classical limit formulas for the translational and rotational enthalpy and entropy for the gas-phase molecules.

One meets much larger problems if the change of the standard free energy is to be calculated for a chemical reaction in solution. For conformational and/or tautomeric transformations, a method was recently proposed for calculating the difference in the internal free energy for the solute molecules and the relative solvation free energy by considering explicit solvent molecules.[11] For such isomeric systems, the molecular mass remains unaltered and if one assumes that the translational energy and entropy depend only on the total mass, then these terms do not change throughout the reaction. For the rotational terms, the classical expression probably represents only an approximation, but if the moments of inertia do not change dramatically, a cancellation in the rotational energy and entropy may be expected. Alternatively, the vibrational frequencies change remarkably even for a tautomeric process; thus, they have to be calculated in solvent media.

However, in a general chemical reaction of the type A + B → C + (D + ···), no cancellation of the translational and rotational terms can be assumed. The masses of the reactant and product molecules are different, and the moments of inertia change as well. Accordingly, there remains a problem of how to most appropriately calculate the corresponding contributions to the relative standard potentials. This question was briefly discussed recently by Ho et al.[53] who, referring to Ben-Nun and Levine,[54] expressed their opinion that "the ideal gas partition functions, particularly the translational and rotational contributions, are unlikely to be valid in solution". The present authors agree with this conclusion, and attempts have been made for calculating free energy changes only for the proton-jump in isomeric complexes.

Table 2 shows that the net energy change upon the formation of the acetate–protonated-amine complex from their infinitely separated constituents is a balance of the negative ΔE_{int} and the positive ΔG(solv) term. The sum is negative in all four cases. The net energy of ΔE_{int} + ΔG(solv) changes

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Table 2. Calculated Energies of Formation for the Acetate···Protonated Amine Complexes from Separated Ions in Aqueous Solution[a]

	$^+H_3NCH_3$	$^+H_2N(CH_3)_2$	$^+HN(CH_3)_3$	$^+N(CH_3)_4$
		ΔE_{int}		
MP2/aug-cc-pvdz	−135.1	−132.1	−130.4	−101.4
ε = 15[b]	−134.8	−131.8	−130.2	−101.6
ε = 5[b]	−134.1	−131.0	−129.6	−101.5
MP2/aug-cc-pvtz	−135.1	−132.0	−130.2	−100.9
$MP2_{CBS}$	−135.1	−131.9	−130.0	−100.7
CCSD(T)	−135.1	−132.0	−130.2	−101.7
$CCSD(T)_{CBS}$	−135.1	−131.8	−129.8	−100.9
B97D/aug-cc-pvtz	−133.5	−128.7	−126.1	
$B97D_{CBS}$	−133.3	−128.5	−125.9	
		ΔG(solv)		
ΔG_{elst}				
MP2[c]	119.9	115.8	112.0	95.3
ε = 15[b]	113.0	108.8	105.5	90.6
ε = 5[b]	96.4	92.6	89.9	78.2
B97D	118.5	113.3	109.8	
ΔG_{drc}				
MP2	0.7	1.0	1.0	1.9
ε = 5, 15[d]	0.7	0.9	1.0	1.7
B97D	0.7	1.1	1.2	
		ΔE_{tot}		
$CCSD(T)_{CBS}$[e]	−14.6	−15.0	−16.8	−3.7
ε = 15[b]	−21.1	−22.1	−23.8	−9.3
ε = 5[b]	−37.1	−37.5	−38.7	−21.6
$B97D_{CBS}$	−14.1	−14.1	−14.9	

[a]Energies in kcal/mol. [b]Obtained in single-point calculation at the IEF-PCM/MP2/aug-cc-pvdz optimized geometry and level in aqueous solution. [c]Calculated at the IEF-PCM/MP2/aug-cc-pvtz level in aqueous solution. [d]The environment was characterized by a "model acetone solvent" with ε set artificially to 5 and 15 in the present study. Then, the ΔG_{drc} term does not change with varying dielectric constants in single-point calculations with fixed solute geometry in the cavity. [e]$\Delta E_{tot} = \Delta E_{int}(CCSD(T)_{CBS}) + \Delta G_{elst} + \Delta G_{drc}$ in aqueous solution.

from −14.6 to −16.8 kcal/mol with partners of methyl-, dimethyl-, and trimethyl amine from $CCSD(T)_{CBS}$ calculations. $\Delta E_{int} + \Delta G$(solv) decreases suddenly down to −3.7 kcal/mol through the interaction with the quat.

Inspection of the individual terms reveals that the $CCSD(T)_{CBS}$ ΔE_{int} becomes less negative by 5.4 kcal/mol if the number of the methyl substituents increases to three. In the case of a fourth methyl substituent, however, ΔE_{int} decreases (in absolute value) by another 29 kcal/mol. This change has a dramatic effect in the $\Delta E_{int} + \Delta G$(solv) series. The considerably smaller energy stabilization for this system upon complex formation has been interpreted so that no strong hydrogen bond can be formed in the acetate−quaternary ammonium complex. The C···N distance increases by about 1 Å compared with cases involving other cations (Table 1), followed by an increased separation of the centers of the negative and positive charges in the complex.

The ΔG(solv) terms gradually decrease for the four cations considered. The finding that the $\Delta E_{int} + \Delta G$(solv) sum is negative with the methyl to trimethyl partners means that the less negative internal energy stabilization due to the interaction of the partners is entailed with an even larger decrease in the solvent effects. For the quaternary amine complex, ΔE_{int} and the ΔG(solv) terms are much closer to each other in absolute value than in the other three cases, resulting in ΔE_{tot} of only −3.7 kcal/mol. The $B97D_{CBS}$ association energies for the three complexes are slightly less negative than those from the ab initio studies. The basis set superposition error is of only 0.2 kcal/mol from B97D/aug-cc-pvtz calculations, corresponding to less than 0.2%.

The above results indicate that the formation of the complex from their separated ionic constituents is favorable in all four cases. The question emerges, however, whether the complex saves its ion-pair character or a proton jumps over from the cation to the acetate ion, resulting in the formation of the neutral, hydrogen-bonded form. In our previous study,[7] we concluded that the neutral form is more stable than the ion-pair in a low-dielectric-constant medium, although larger ε supports an increasing ionic fraction.

Formation of the Neutral Complex. When the two elements of the complexes under scrutiny in the present study are separated in aqueous solution, each of the constituents is expected to take an ionic form by deprotonation (acid) and protonation (base). As they approach each other, they can partially desolvate and form a complex (see the previous section where this process is largely favored energetically), and the neutral and ion-pair forms of the hydrogen-bonded complexes are identified on the basis of the position of the critical proton. Whether it resides at the acid site or on the base, we distinguish the two tautomers. Although the geometry of the complex changes upon the shift of the proton, the total mass is still conserved and only small variations are expected in the moments of inertia. Thus, the physical−chemical process to be followed in this case is the formation of a complex from two largely separated ionic species and consideration whether a proton-jump from the protonated amine to the acetate ion would stabilize the system. If the

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complex dissociates, the ion-pair form would be regained in either case.

As discussed above, calculation of the relative free energy for tautomers is promising even in solution. Table 3 shows the relative energy/free energy components for the ion-pair relative to the hydrogen-bonded acetic acid−neutral amine tautomer. The relative internal energy, ΔE_{int}, is always positive. The importance of the high-level energy calculations is revealed by data in Table 3. The relative CCSD(T)−MP2/aug-cc-pvdz term is always positive, amounting to 0.7 kcal/mol in the case of the acetic acid−methylamine complex. The 0.7 kcal/mol post-MP2 correction is a large fraction of the overall 1.9 kcal/mol ΔG_{tot}. The correction decreases to 0.1 kcal/mol for the trimethylamine complex. The relative $MP2_{CBS}$ corrections are about 0.2 kcal/mol compared to the large-basis-set MP2/aug-cc-pvtz values. The $B97D_{CBS}$ ΔE_{int} value is smaller by 2.1 kcal/mol than the $CCSD(T)_{CBS}$ value for the methylamine complex but is larger by 0.5 kcal/mol than the relative $CCSD(T)_{CBS}$ internal energy when the base partner is trimethylamine.

Table 3. Free Energies of the Ion Pairs Relative to the Neutral Hydrogen-Bonded Form in Aqueous Solution as Calculated at the IEF-PCM/MP2/aug-cc-pvdz and IEF-PCM/B97D/aug-cc-pvtz Geometries[a]

	$^+H_3NCH_3$	$^+H_2N(CH_3)_2$	$^+HN(CH_3)_3$
	ΔE_{int}		
MP2/aug-cc-pvdz	12.2	8.5	6.9
MP2/aug-cc-pvtz	12.6	9.0	7.3
$MP2_{CBS}$	12.8	9.2	7.5
CCSD(T)	12.9	9.0	7.0
ε = 15	12.1[b]		
ε = 5	9.7[b]		
$CCSD(T)_{CBS}$	13.5	9.7	7.8
B97D/aug-cc-pvtz[c]	11.3	9.5	8.2
$B97D_{CBS}$	11.4	9.7	8.3
	ΔG(solv)		
ΔG_{elst}	−12.9[d]	−10.0	−8.7
ε = 15	−10.9[b]		
ε = 5	−6.8[b]		
B97D[c]	−10.5	−9.5	−8.7
ΔG_{drc}	−0.2	−0.1	−0.1
B97D[c]	0.0	0.1	−0.1
ΔG_{th}[e]	1.5	1.6	1.7
	ΔG_{tot}		
$CCSD(T)_{CBS}$[f]	1.9	1.2	0.7
B97D[g]	2.3	1.9	1.2

[a]Values in kcal/mol. [b]Reference 7. [c]Calculated with IEF-PCM/B97D/aug-cc-pvtz optimized geometry. [d]Single-point IEF-PCM/MP2/aug-cc-pvtz value. [e]After neglecting the lowest real and imaginary frequencies. See the text. [f]$\Delta G_{tot} = \Delta E_{int}(CCSD(T)_{CBS}) + \Delta G$(solv, MP2) + ΔG_{th}. [g]$\Delta G_{tot} = \Delta E_{int}(B97D_{CBS}) + \Delta G$(solv, B97D) + ΔG_{th}.

ΔG(solv) varies in the −8.8 to −13.1 kcal/mol range from IEF-PCM/MP2/aug-cc-pvtz calculations in water. The B97D/ΔG(solv) values vary only in the −8.8 to −10.5 kcal/range. In both series, ΔG(solv) is the most negative for the methylamine complex. From previous ab initio results for this latter species, the CCSD(T) relative energy increases monotonically and ΔG_{elst} becomes more negative in parallel in the ε = 5, 15, 78.39 series.

For calculating ΔG_{tot}, estimation of the considerable relative thermal correction, ΔG_{th}, is necessary. All-positive frequencies were obtained only for the optimized, neutral acetic acid−dimethylamine and acetic acid−trimethylamine complexes, whereas 22i−45i imaginary frequencies were obtained for other systems. The imaginary frequencies were always related to the methyl torsion of the acetic acid component. The present study represents the simplest model for calculating the interaction of an amine ligand with a carboxylic side-chain appearing in a protein. However, the corresponding side-chains in proteins are those of Asp and Glu with a $-CH_2-COO(H)$ moiety. In our previous study, the missing torsional frequencies were replaced by the corresponding C−C−C−O torsions in the propionic acid. The values for the neutral and ionized species were practically equal (50.5 and 50.6 cm^{-1}) at ε = 15. Thus, neglect of the vibrational contribution due to the lowest frequencies through the calculation of the relative thermal correction seems to be an acceptable approximation and it was applied here.

The neutral-form/ion-pair ratio depends ultimately on $\Delta G_{tot} = \Delta E_{int} + \Delta G$(solv) + ΔG_{th}. Since this value is positive for all acetic acid complexes with methyl, dimethyl, and trimethylamine partners, the neutral, hydrogen-bonded rather than ion-pair forms have been predicted to be more stable on the basis of the IEF-PCM continuum solvent approach. The ab initio ΔG_{tot} values are 1.9, 1.2, and 0.7 kcal/mol for the series above. The corresponding B97D values are slightly larger, 2.3, 1.9, and 1.2 kcal/mol. The relatively small difference in ΔG_{tot} for, e.g., the methylamine complex is the consequence of the only 0.5 kcal/mol difference in $\Delta E_{int} + \Delta G$(solv) values: 13.5−13.1 and 11.4−10.5, respectively, despite the 2.1−2.6 kcal/mol differences in the individual terms. Thus, ΔE_{int} and ΔG(solv) changes in absolute value in parallel, allowing for similar predicted ΔG_{tot} values from ab initio and DFT studies.

Table 3 suggests, however, that correct estimation of the $\Delta E_{elst} = \Delta G_{elst}$ term, as the leading term in ΔG(solv), is a key issue in cases of sensitive equilibria. PCM-type calculations apply through-space polarization of the solute by the environment (polarizable continuum dielectric) and do not take into consideration the solvent effects through the formation of hydrogen bond(s) between the solute and a protic solvent, like water. Furthermore, the obtained value of ΔG_{elst} has proven to depend on the level applied in the IEF-PCM calculations. When the B3LYP/aug-cc-pvtz wave function was used for determining ΔG_{elst} at the MP2/aug-cc-pvdz optimized geometry, the estimated values were 0.60−0.85 kcal/mol less negative than those from in-solution single-point MP2/aug-cc-pvtz calculations, thus increasing the positive ΔG_{tot} in the studied cases. An even larger departure from ΔG_{elst} was also noticed on the basis of the B97D/aug-cc-pvtz estimation, although with modified geometries for the complexes. The sensitive balance of ΔE_{int} and ΔG(solv) calls for the calculation of this latter term from another source, where solute−solvent hydrogen bonds are taken into account by considering explicit solvent molecules. Monte Carlo simulations below have been performed to meet this goal.

Monte Carlo Simulations in Aqueous Solution. Table 4 summarizes the calculated ΔG(solv) values for the neutral acid···amine to acetate···protonated amine transformations by considering explicit (TIP4P) water molecules. Forward and backward simulations agree very well. Even admitting some uncertainty for ΔG(solv) calculated with and without a reaction field and using different ICUT options for the infinitely dilute model, the general result is that the MC value is much more negative than about −10 to −13 kcal/mol for the

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Table 4. Monte Carlo Solvation Free Energies and Calculated Total Free Energy for the Ion Pair Relative to the Neutral Complex[a]

	water		chloroform	
	ΔG(solv)/MC	ΔG_{tot}	ΔG(solv)/MC	ΔG_{tot}
	$CH_3COO^-\cdots{}^+H_3NCH_3$			
infinitely dilute, ICUT = 2	−22.6 ± 0.1[b]		−2.9 ± 0.0	
	−22.3 ± 0.1[b]			
with reaction field, ICUT = 0	−19.8 ± 0.1[c]		−2.8 ± 0.0	6.8[d]
	−19.7 ± 0.1[c]	−4.7 ± 0.1		
0.11 molar, Ewald, ICUT = 2	−16.0 ± 0.1[b]	−1.0 ± 0.1		
B97D	−13.5 ± 0.1	−0.6 ± 0.1		
0.11 molar + NaCl, Ewald, ICUT = 2	−15.8 ± 0.3[b]	−0.8 ± 0.3		
	$CH_3COO^-\cdots{}^+H_2N(CH_3)_2$			
with reaction field, ICUT = 0	−17.1 ± 0.0	−5.8 ± 0.0		
0.11 molar, Ewald, ICUT = 2	−13.7 ± 0.0	−2.4 ± 0.0		
B97D	−12.5 ± 0.1	−1.3 ± 0.1		
	$CH_3COO^-\cdots{}^+HN(CH_3)_3$			
0.11 molar, Ewald, ICUT = 2	−11.4 ± 0.0	−2.0 ± 0.0		
B97D	−11.4 ± 0.0	−1.4 ± 0.0		
0.11 molar + NaCl, Ewald, ICUT = 2	−11.7 ± 0.0	−2.2 ± 0.0		
	$CH_3COO^-\cdots 2H_2O\cdots{}^+H_3NCH_3$			
with reaction field, ICUT = 0			−4.1 ± 0.0	~6.6

[a]Relative free energies in kcal/mol. Standard deviations less than 0.05 kcal/mol are rounded to 0.0. B97D results were obtained by considering the corresponding optimized geometries and the B97D/aug-cc-pvtz derived charges. For the ICUT cutoff methodology, see the text. $\Delta G_{tot} = \Delta E_{int}(CCSD(T)_{CBS}) + \Delta G(solv)/MC + \Delta G_{th}$, corresponding data from Table 3 (water) and Table 7 (chloroform). [b]Forward simulation. [c]Backward simulation. [d]Calculated on the basis of $\Delta E_{int}(CCSD(T)) + \Delta G_{th}$ = 9.65 kcal/mol with ε = 5 (ref 7).

$CH_3COOH\cdots NH_2CH_3$ to $CH_3COO^-\cdots{}^+H_3NCH_3$ transformation in Table 3. Considering only −19.7 kcal/mol for ΔG(solv)/MC from Table 4, the ΔG_{tot} term becomes strongly negative, favoring the ion-pair form. In this respect, it does not matter whether the contact ion-pair is the thermodynamically most stable form or perhaps water-separated but near ions are even more favored. The main conclusion is that the optimized neutral complex is less stable than a contact ion-pair in aqueous solution; thus, no proton jump to the acetate emerges here.

If the long-range electrostatic correction is calculated by Ewald summation, then the model corresponds to a 0.11 molar solution of the complex. For this model and using the ICUT = 2 cutoff method, ΔG(solv)/MC is less negative by about 6−7 kcal/mol than that for the infinitely dilute model. The presence of Na^+/Cl^- ions, simulating a nearly isotonic saline solution, would not change the results significantly. The sensitivity of the tautomeric ratio to the solution concentration is in accord with the findings of Moriyasu et al.[55] and De Oliveria et al.[56] A considerable decrease in the solvent effect was predicted upon MC/FEP calculation when the B97D optimized geometry and parametrization were used. Since, however, ΔE_{int} also decreased at this level of theory (see Table 3), the predicted ΔG_{tot} is −0.6 ± 0.1 kcal/mol. This free energy difference allows for the presence of up to 34% neutral complex, as calculated at the 95% probability (2SD) level.

Recent studies for protonated amine···chloride systems indicated that the minima of the potential of mean force (pmf) curves correspond to a contact ion-pair, but the coordination numbers for the cation by the anion are small. An MC study for the protonated, nonaromatic six-member rings with two nitrogens found that the chloride anion oscillates between positions in direct contact with the cation and when the ion-pair assumes a water-separated structure.[10] Molecular dynamics and MC studies for aqueous trimethyl- and dimethylammonium chloride solutions led to similar conclusions.[57,58]

Charge transfer was not allowed in any of these studies. Tables S1 and S2 (Supporting Information) indicate, however, charge transfers of 0.13−0.20 charge units for the contact ion-pairs at their PCM optimized geometry in aqueous solution, and about 0.24 units in chloroform. Even larger values were obtained for the neutral complexes. A correct pmf curve should allow charge transfer for water-separated elements, as well. The first step toward ion dissociation is the formation of a water-separated ion-pair form. For an acetate complex, the $CH_3COO^-\cdots 2H_2O\cdots{}^+H_3NCH_3$ water-bridged ion-pair corresponds to a local energy minimum both in water and in chloroform (Scheme 2, Table 1). Table S3 (Supporting Information) indicates transfer of about 0.2 charge units from the anion to the cation even in the presence of two water molecules comprising a hydrogen-bonded network.

The charge transfer between more separated ions should be smaller, but it could be explored only by considering partially optimized geometries when the C···N separation is fixed between, e.g., 3.15 and 4.15 Å, and varying their distance by 0.2 Å for obtaining reference structures for the pmf. A quick single-point calculation with R(C···N) set to 3.85 Å showed that the charge transfer is still 0.05 atomic units. The computational cost for the calculation of such a pmf would be large because of the slow convergence in IEF-PCM optimizations. Furthermore, calculation of ΔG_{th} for non-energy-minimum structures raises another problem. Nonetheless, it can be concluded on the basis of the present MC simulations that the ion-pair form of the $CH_3COO^-\cdots{}^+H_3NCH_3$ complex is maintained as the contact form in aqueous solution.

MC simulations for exploring the possibility of the proton jump for the acetic acid···di- and trimethylamine systems (Table 4) also resulted in much more negative ΔG(solv) values than the counterparts in Table 3. Thus, for these two complexes, the ion-pair form is the stable tautomer in solution and is present in at least 86% (ΔG_{tot} = −1.3 + 0.2 kcal/mol, the B97D value at the 95% probability upper limit).

Solution Structure. The solution structure in the first solvation shell of the solute molecules has been characterized by means of solute−solvent radial[59] and pair-energy distribution (pedf) functions.[11,33,38,42] Combination of the calculated coordination numbers and the numbers of strongly bound water molecules (n_{SB}) in Table 5 helps interpret the solute−solvent hydrogen-bond pattern. The discussion below refers to the more complete set of results obtained by using the MP2/aug-cc-pvdz geometries and the B3LYP/aug-cc-pvtz charge parametrization. The generally small deviations upon the B97D calculations leave the final conclusions unaltered.

The carbonyl oxygen/water hydrogen coordination number, $=O/H_w$ (corresponding to O^-/H_w in the ion-pair), is always about 2. The hydroxyl oxygen, O/H_w (corresponding to the

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Table 5. Coordination Numbers and Numbers of Strongly Bound Water Molecules (n_{SB})[a]

	=O/ H_w	O/ H_w	N/ H_w	NH/ O_w	Na^+/ O_w	Cl^-/ H_w	n_{SB}
			$CH_3COOH\cdots H_2NCH_3$				
0.11 M	2.0	1.2	0.01	0.7[b]			2.8
B97D	2.2	1.1	0.01	0.8			3.5
0.11 M + Na^+/ Cl^-	2.0	1.2	0.01	0.7[b]	6.2	7.3	15.3
			$CH_3COO^-\cdots{}^+H_3NCH_3$				
0.11 M	2.3	2.1		1.1[b]			6.3
B97D	2.6	2.0		1.2			6.4
0.11 M + Na^+/ Cl^-	2.4	1.9		1.2[b]	6.2	7.3	19.1
			$CH_3COOH\cdots HN(CH_3)_2$				
0.11 M	2.1	1.1	0[c]	0.6			3.1
B97D	2.3	1.2		0.8			3.3
			$CH_3COO^-\cdots{}^+H_2N(CH_3)_2$				
0.11 M	2.4	1.9		1.0			4.7
B97D	2.2	1.8		1.1			4.6
			$CH_3COOH\cdots N(CH_3)_3$				
0.11 M	2.0	1.2	0[c]				3.2
B97D	2.0	1.3					3.3
0.11 M + Na^+/ Cl^-	2.0	1.4	0[c]		4.9	5.4	12.7
			$CH_3COO^-\cdots{}^+HN(CH_3)_3$				
0.11 M	2.0	2.0		0[c]			3.9
B97D	2.2	1.9					4.0
0.11 M + Na^+/ Cl^-	2.1	2.0		0[c]	6.4	7.0	16.9

[a]Coordination numbers were calculated by integrating the rdf's up to 2.50 Å for the solute/water atoms, and up to 3.35 and 3.05 Å for Na^+/ O_w and Cl^-/H_w, respectively. [b]Average value for the H(N) atoms not in a hydrogen bond with the acid. [c]The coordination number is less than 0.01 at R = 2.50 Å.

other O^-/H_w in the ion-pair), is coordinated by about one hydrogen with the water solvent in the neutral acid, and by about 2 in the form O^-/H_w. These coordination numbers allow formation of up to one and two hydrogen bonds with the solvent, respectively. No coordination number for the tautomeric proton, H_t, by a water oxygen is indicated in the table. This proton is always deeply buried in any complex both in its neutral and ion-pair forms, and no water oxygen can approach it in order to form a hydrogen bond. Instead, this proton forms a hydrogen bond within the complex either in the form of $OH_t\cdots N$ or $O^-\cdots{}^+H_tN$. Similarly, the basic site of the neutral amine is not reachable by water hydrogens, as revealed from the nearly always zero N/H_w value.

If there is an NH proton on the outskirts of the complex, it is coordinated by water oxygens. The NH/O_w values are less than 1 for the neutral complexes, since the amine hydrogen is a weak hydrogen-bond donor. In contrast, NH/O_w is about 1 for each N−H bond in the ionic species, indicating that the protonated amine is a strong hydrogen-bond donor. The NH/O_w value is zero even for a protonated amine species in the $CH_3COO^-\cdots{}^+HN(CH_3)_3$ complex, because this N−H bond is involved in the intermolecular hydrogen bond.

Coordination numbers provide an upper limit for the possible number of the solute−solvent hydrogen bonds at the given site. A water molecule involved in hydrogen bonding to the solute is a relatively strongly bound molecule, contributing to the pair-energy distribution function generally with energies more negative than that corresponding to the first minimum. For an example, the pedf's are shown for the acetic acid−methylamine system in Figure 1. Integration of the curve up to its first minimum at about E = −5 kcal/mol provides n_{SB}. (For the specific integration limits, see the figure legend.)

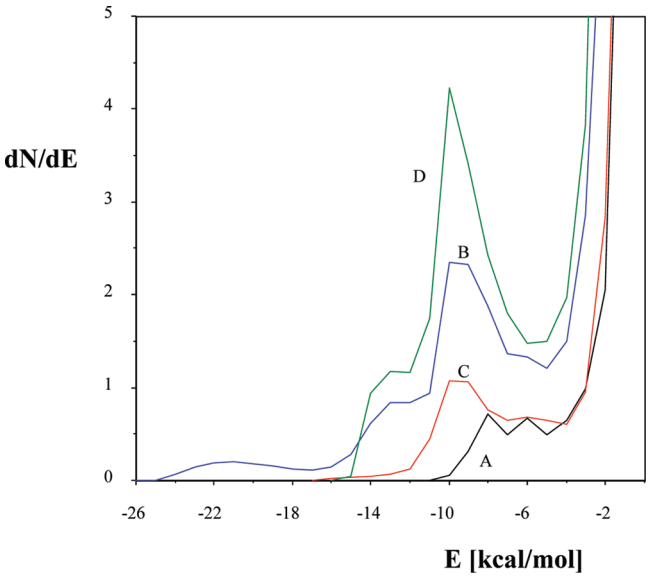

Figure 1. Solute−water pair-energy distribution functions for the 0.11 M acetic acid−methylamine complexes with ICUT = 2 cutoff option and Ewald correction. Integration limits are E = −5 kcal/mol (neutral form, A), −5 kcal/mol (neutral form + Na^+/Cl^-, B), −4 kcal/mol (ion-pair, C), and −5.5 kcal (ion-pair + Na^+/Cl^-, D).

The curves have their maxima in the range of E = −10 to −8 kcal/mol, but the pedf's accounting for the N^+/Cl^- containing models (B and D) clearly indicate a shoulder around $E \approx -13$ kcal/mol. The much higher peak values for these latter curves are the consequence of the presence of the strongly bound water molecules to the sodium and the chloride ions. The large-energy tail of curve B may indicate some sampling problem reflecting long existing strong ion−water association(s). Integration of B up to E = −16 kcal/mol provides, indeed, 1.3 unusually strongly bound water molecules. The effect of this interaction is, however, negligible in the FEP calculations, because its contribution to the total energy considering the reference and perturbed solute structure is nearly equal.

It reveals from Table 5 that the sum of the coordination numbers (scn) is larger than the corresponding n_{SB} value, mainly for the neutral species. In the case of the 0.11 M solution of the neutral acetic acid···methylamine complex, scn = 2 + 1.2 + 0.01 + 2 × 0.7 ≈ 4.6 compared with n_{SB} = 2.8. The difference clearly indicates that the coordination number provides the maximum number of the possible hydrogen bonds, although not every involved water molecule enters such strong solute−solvent interaction, generally with E < −4 kcal/mol (see Figure 1). On the other hand, the solute−water interactions are strong for the ionic species; snc = 6.6 and n_{SB} = 6.3 agree very well for this complex. Snc is slightly larger than n_{SB} both for the neutral and ionic forms of the acetic acid···dimethylamine complex, whereas they are nearly equal for both tautmomers with the trimethylamine partner (Table 5). No general trend has been found for the snc > n_{SB} vs snc ≈ n_{SB} relationship as a function of the chemical structure.

Table 5 provides the calculated Na^+/O_w and Cl^-/H_w coordination numbers, as well, from models mimicking isotonic

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saline solutions. The Cl^-/H_w coordination number was thoroughly analyzed for the dilute dimethylammonium chloride solution, and it was concluded that Cl^-/H_w is about 7.3 for a fully hydrated chloride anion.[58] This coordination number was interpreted as the possibility for forming up to 7−8 (mostly only seven) $Cl^-\cdots H_w$ hydrogen bonds with surrounding water molecules. The value does not necessarily require involvement of different water molecules, although snapshots generally do not show bifurcated water···chloride hydrogen bonds.

If Cl^-/H_w is 7.0−7.3 in Table 5, Na^+/O_w = 6.2−6.4, meaning 6−7 water oxygens around the sodium cation. Snapshots show that no Na^+ is connected to acetic acid/acetate oxygens; thus, if the chloride is fully dissolved, then the sodium ion must also do so. In this case, six to seven water molecules surround a sodium ion and seven to eight waters form hydrogen bonds with the chloride. On the basis of the present analysis, no answer can be given whether one to two water molecules jointly appear in both hydration spheres or there are six to seven and seven to eight distinct water molecules solvating the sodium and chloride ions, respectively. This latter case must take place if the ions are separated by at least 7−8 Å, as snapshots indicate in some cases. A frequent separation is, however, only about 5 Å, which suggests that some water oxygens face the sodium cation while one of their hydrogen atoms would be in a hydrogen-bond relationship to the chloride ion.

In these examples, the Na^+ and Cl^- ions must take at least a solvent separated form. If this condition is not met, the coordination number averaged over 7.5 million configurations must be smaller than the above values considered as the upper limit for the coordination numbers by the present parametrization and modeling technique. For the 0.11 M + Na^+/Cl^- model of the $CH_3COOH\cdots N(CH_3)_3$ complex, Na^+/O_w = 4.9 and Cl^-/H_w = 5.4. This is only possible if one to two water molecules are missing from each individual hydration sphere, and the ions take a contact ion-pair form. Indeed, the last snapshot finds that the Na^+ and Cl^- ions are separated only by 3.06 Å. This is much less than the half of the sum of the sigma parameters as of 4.04 Å,[60] the limit value for the nonrepulsive LJ energy. Such close contacts do not seem to be the prevailing form for the Na^+/Cl^+ ion pair (see the above results) but are energetically allowed and can be maintained even after considering 15 M configurations. The water molecules solvating the two ions must be strongly bound, as concluded from the finding that the difference of the corresponding n_{SB} values calculated with and without NaCl in the systems is generally close to the sum of the Na^+/O_w and Cl^-/H_w coordination numbers.

Complex Formation with 2-Methoxy Acetic Acid. Because of the very time-consuming calculations, the investigation for complex formation with 2-methoxy-acetic acid was limited. In this case, conformational problems emerge in addition to every tautomeric equilibrium, making a complete structural analysis even more demanding. Accordingly, only some energy parameters have been explored for the conformer with an almost eclipsed OCCO moiety and a distorted gauche arrangement along the COCC path (Table 1).

The corresponding MP2 relative energies are less positive by about 1.5 kcal/mol for the 2-methoxy acetic acid−methylamine complex than for the complex with acetic acid. This is a remarkable decrease, whereas ΔG(solv)/PCM remains nearly unaltered (using the MP2 value). An estimate for ΔG_{tot} may be made if one applies $\Delta E_{int}(MP2_{CBS})$ and takes all other terms (post-MP2 correction, ΔG_{th}, and ΔG(solv)/MC) as calculated for the complex with the acetic acid partner obtaining 11.3 + 0.7 + 1.5 − 15.8 = −2.3 kcal/mol. This relative total free energy predicts the overwhelming amount of the ion-pair tautomer in aqueous solution or on the surface of a protein. The conclusion cannot be considered as a surprise provided that the pK_a is 3.54 for 2-methoxy acetic acid compared with 4.79 for acetic acid. The stronger acidic character of the 2-methoxy derivative explains its larger proton donating ability and then the increased relative stability of the ion-pair form in the complex.

Complex in a Receptor Cavity. The possible proton jump leading to tautomerization of the acetic acid−methylamine complex was studied in a nonpolar environment, as well. Formerly, $\Delta E_{int}(MP2_{CBS}) + \Delta G(solv)/PCM = 9.7 - 6.8 = 2.9$ kcal/mol was calculated for the contact $CH_3COO^-\cdots{}^+H_3NCH_3$ pair in a low polarity dielectric with $\varepsilon = 5$.[7] The present, MC-estimated ΔG(solv) is about −2.9 kcal/mol in chloroform (Table 4); thus, the combined results predict a proton jump resulting nearly exclusively in formation of the neutral pair in this solvent.

It is also conceivable, however, that there are a few water molecules hydrating the complex in certain receptor environments, and the ion-pair does not adopt a contact form. In our model for the binding within a receptor cavity, two water molecules act as hydrogen donors to the carboxylate group and these waters act as hydrogen bond acceptors toward the proton(s) of the base partner (Scheme 2, structures 4a and 4b). The model seems reasonable on the basis of the discussed structure of the first hydration shell for the ion-pair in aqueous solution. After a possible proton jump, one of the water molecules will behave as an acceptor in the O═C—O—H···OH_2 moiety, and will act as a hydrogen bond donor to the neutral amine nitrogen as HOH···$NH_xR_{(3-x)}$. The geometry parameters and the relative free energy terms calculated for the $CH_3COOH\cdots 2H_2O\cdots NH_2CH_3$ to $CH_3COO^-\cdots 2H_2O\cdots{}^+H_3NCH_3$ transformation are provided in Table 7.

Table 6. Calculated Energy for the 2-Methoxy-Acetate···Methylammonium Ion Pair Relative to the Hydrogen-Bonded Form in Aqueous Solution[a]

	MP2/aug-cc-pvdz	MP2/aug-cc-pvtz[b]	$MP2_{CBS}$
ΔE_{int}	10.6	11.1	11.3
ΔG(solv)			
ΔG_{elst}	−13.0	−13.0	
		−12.1[c]	
ΔG_{drc}	−0.1		
ΔE_{tot}[d]	−2.6	−2.1	−1.9

[a]Energies in kcal/mol. [b]Single-point calculation at the IEF-PCM/MP2/aug-cc-pvdz optimized geometry. [c]IEF-PCM/B3LYP/aug-cc-pvtz value. [d]$\Delta E_{tot} = \Delta E_{int}(MP2) + \Delta G_{elst} + \Delta G_{drc}$.

Geometric results indicate four hydrogen bonds between the two water molecules and the organic partners. It is noteworthy that, due to the increased C···N distance, no direct hydrogen bond is feasible between the acetic acid and the methylamine molecules in any tautomeric complex. The relative $CCSD(T)_{CBS}$ energy for the ion-pair form is 8.7 kcal/mol, whereas the calculated ΔG(solv)/PCM indicates a very subtle balance. Frequency calculations gave 30i and 50i imaginary frequencies for the acetyl methyl vibration. Even worse, another imaginary frequency of 13i was found for the ion-pair. Presumably, both of them were the consequence of the very flat potential energy surface around the minimum such that the Berny optimization

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Table 7. MP2/aug-cc-pvdz Hydrogen-Bond Parameters and Free Energy Contributions of the Ion Pair + Two Waters Relative to the Neutral Hydrogen-Bonded Form + Two Waters in Chloroform[a]

		C···N[a]	O···N[b]	O/N···H[b]	O···H···N
$CH_3COO^-\cdots 2H_2O\cdots {}^+H_3NCH_3$		4.23	3.59		
$O_{wat1}\cdots {}^+H_1(N)$				1.68	165
$O_{wat2}\cdots {}^+H_2(N)$				1.73	158
$H_{wat1}\cdots {}^-O_1$				1.60	176
$H_{wat2}\cdots {}^-O_2$				1.62	170
$CH_3COOH\cdots 2H_2O\cdots NH_2CH_3$		4.36	3.91		
$O_{wat1}\cdots H(O)$				1.61	172
$O_{wat2}\cdots H(N)$				2.10	145
$H_{wat1}\cdots N$				1.70	170
$H_{wat2}\cdots O=$				1.87	180
	ΔE_{int}	ΔE_{elst}[b]	ΔG_{drc}	ΔG_{th}	ΔG_{tot}
MP2/aug-cc-pvdz	7.4		−0.3	~ 2[c]	
MP2/aug-cc-pvtz	7.5	−8.6			
$MP2_{CBS}$	7.5				
CCSD(T)	8.5				
$CCSD(T)_{CBS}$	8.7				~1.8

[a]IEF/PCM/MP2/aug-cc-pvdz geometry optimization. Distances in Å, angles in degrees, energies in kcal/mol. [b]From IEF-PCM/MP2/aug-cc-pvtz single-point calculations. [c]Calculated by neglecting the lowest two vibrations.

with default parametrization stopped before reaching the correct minima. By discarding the two lowest frequencies for each system (a more detailed argument for this process is provided as Supporting Information), an approximate ΔG_{th} of about 2 kcal/mol was assigned, and a ΔG_{tot}/PCM of about 1.8 kcal/mol was calculated.

The ΔG(solv)/MC value is, however, −4.1 kcal/mol (Table 4) compared with ΔG(solv)/PCM of −8.9 kcal/mol. By accepting the ΔG(solv)/MC value, the estimated ΔG_{tot}/MC for the ion-pair tautomer is 8.7 − 4.1 + 2 ≈ 6.6 kcal/mol. This is a convincingly large positive value despite several tenths of a kcal/mol uncertainty regarding ΔG_{th}.

As a conclusion, ΔG_{tot}/MC definitely predicts the proton jump from the methylammonium cation to the acetate ion in the above model of the ligand binding in a receptor cavity. The present study does not follow the mechanism of this process. Only the total relative free energy has been calculated, which, however, suffices to identify the prevailing tautomeric form in a model protein environment.

The above result helps answer the question raised in the Introduction: Is the ionic interaction important between the carboxylate side-chain and the ligand in the binding cavity of muscarinic receptors? The large relative stability for the neutral form compared with the ionic tautomer suggests that, if the proton is mobile on the ligand, then it favorably jumps over to the $-COO^-$ group. Evolution of Ach as the natural muscarinic agonist by virtue of only its ability to maintain the ion-pair tautomeric form due to its permanent cationic structure remains open to speculation. Implicit in this possibility is that the ionized Asp/Glu side-chain is regarded as a structural requirement for receptor activation.

CONCLUSION

Association energy of an acetate ion and a simple cationic amine with one to three methyl groups was calculated in the range of −14 to −17 kcal/mol by the IEF-PCM method at the CCSD(T)/CBS and B97D/CBS levels in aqueous solution. The association energy with the quaternary amine is only −3.7 kcal/mol in the absence of an intermolecular hydrogen bond and due to the increased C(carboxylate)···N distance in the complex.

The possible proton jump from the cation to the acetate ion was studied for three protonated amines in aqueous solution. The IEF-PCM method predicts a subtle balance of the relative internal and solvation free energy terms. Using Monte Carlo simulations considering explicit water models, the derived ΔG_{tot} predicts the predominance of the ion-pair rather than the neutral tautomeric form for all three considered complexes. Calculations for the 2-methoxy-acetic acid···methylamine complex also predict the stability of the ionic pair in aqueous solution.

A model for the complex within a binding cavity of a protein was studied by considering two strongly bound water molecules on the contact surface, and a low dielectric environment was mimicked by considering chloroform solutions. When the solvent effects were calculated by means of Monte Carlo simulations, enhanced stability of the neutral complex was predicted. This result would suggest that Ach, an inherent cation by its structure, may have developed as the natural agonist of muscarinic receptors because only a quaternary ammonium can preserve the ion-pair form with a carboxylate side-chain in a protein cavity. This is then also in line with the prevailing hypothesis that the deprotonated form of an Asp/Glu side-chain is a structural requirement for receptor activation.

ASSOCIATED CONTENT

Supporting Information

Atomic charges applied in Monte Carlo simulations are provided in Tables S1−S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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