Theoretical Analyses of the Tautomeric and Conformational Equilibria of Histamine and ($\alpha R,\beta S$)-α,β-Dimethylhistamine in the Gas Phase and Aqueous Solution

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Abstract: Ab initio calculations in the gas phase and Monte Carlo simulations using the statistical pertubation method in aqueous solution have been carried out to study the tautomeric/conformational equilibria of histamine and ($\alpha R,\beta S$)-α,β-dimethylhistamine in both the neutral and protonated forms. The two most stable gas-phase conformers of the neutral histamine molecule are gauche structures stabilized by intramolecular hydrogen bonds. The gauche 1H tautomer is more stable than the gauche 3H structure by 1.7–1.8 kcal/mol at the QCISD/6-31G*//HF/6-31G* and MP2/6-311++G**//HF/6-31G* levels after considering frequency dependent corrections for the free energy at 298 K. Trans conformers at these levels are higher in free energy than gauche 1H by 2.3–3.7 kcal/mol. For the monocations protonated at the side chain the second most stable trans 3H form is higher in free energy by 13 kcal/mol than the most stable gauche 3H structure. The relative free energies of the protonated g1H and t1H tautomers are 19–25 kcal/mol. ($\alpha R,\beta S$)-α,β-Dimethylation of histamine has an effect mainly on the equilibrium geometries leaving the relative energies close to those obtained for histamine. For the equilibrium mixture of the neutral forms (existing at pH > 9–10 in aqueous solution) 87% trans and 13% gauche conformers were calculated for histamine. The preference for the trans over the gauche conformations may be attributed to the larger number of polar sites opened for hydration in aqueous solution. In the monocationic form prevailing under physiological conditions at pH = 7.4, the gauche 3H tautomer is more stable than the trans 1H structure by about 0.4 kcal/mol leading to 64% gauche and 36% trans conformers. This result is in good agreement with previously reported NMR results in solution at pD = 7.9 that predicted 45% trans form. For both the histamine and the ($\alpha R,\beta S$)-α,β-dimethylhistamine solutes the gauche 3H solute is more stable than the trans 3H conformer by about 2 kcal/mol. The theoretical calculations highlight the balance of the internal stabilization and the solvent effect. Solution structure analysis provides rationale for the energy changes in the tautomerization processes and conformational changes upon solvation. The results are discussed in relationship to existing models for histamine receptor activation.

Introduction

Conformational analysis and energy calculations in aqueous solution for molecules with possible intramolecular hydrogen bonds represent a formidable task for theoretical studies. There is a competition in these systems between forces to create intramolecular hydrogen bond(s) and those to form hydrogen bonds to the water solvent. Recent calculations on 1,2-ethanediol[1] and 2-OH benzoic acid[2] show that ab initio calculations in the gas phase augmented with Monte Carlo simulations in water can give answers referred to the relative free energies of the conformers in solution, and a reasonable characterization of solution structure in the first hydration shells of the polar groups is possible.

Histamine, 2-(4(5)-imidazolyl)ethylamine, with two sterically close polar groups also belongs to the class of molecules able to form intramolecular hydrogen bonds (Figure 1). The molecule has two basic centers: the side-chain NH_2 group (pK_a = 9.40) and the basic ring nitrogen (pK_a = 5.80).[3a] In aqueous solution at pH = 7.4 it is estimated that almost 97% of the solute molecules are in the monocation form, protonated at the amine group.[4] At lower pH's consideration of the dication form, the structure protonated both in the ring and at the side chain, also becomes important. The monocation and the neutral forms allow different intramolecular hydrogen bonds in the gauche conformations. These are, however, disrupted in the extended, trans forms. Depending on the position of the ring hydrogen connected to one of the ring nitrogens, there are two possible tautomers. Thus, histamine exhibits a complicated system of different tautomeric, conformational, and protonated states in aqueous solution.

The paramount interest in histamine is due to its important role in biological systems.[3b] It stimulates, acting *via* H_1 receptors, the contraction of many smooth muscles in the gut, the bronchi, and the uterus. Histamine also has an important physiological role in regulating the secretion of acid in stomach, acting on a different receptor characterized and defined as the H_2 receptor. The more recently characterized[5a,b] H_3 receptor modulates the synthesis and release of histamine in the brain and some peripheral tissues. $\alpha(R)$-Methylhistamine was discerned to be a highly selective agonist for the H_3 receptor.[5b] Subsequently, Lipp et al.[6]

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found that a special isomer of the dimethyl derivative, the (αR,βS)-α,β-dimethylhistamine is also a highly potent H_3 agonist. Due to its common pharmacological action at the H_3 receptor and its structural similarity to histamine in the intramolecular hydrogen bond forming capability, this dimethyl derivative is also investigated in the present study.

Histamine and (αR,βS)-α,β-dimethylhistamine have been investigated by X-ray crystallography. The neutral,[7] monocationic,[8] and dicationic[9] histamine structures were all found in the extended, trans conformation (for some computed structures see Figure 2). On the contrary, the structurally related L-histidine hydrochloride salt[10] and the neutral L-histidine zwitterion[11] take the gauche arrangement with an intramolecular hydrogen bond in the latter. Dimethylhistamine in the dication form[6] was found to adopt a gauche arrangement.

Histamine has also widely been investigated using different theoretical methods[12] while only a single molecular mechanics study has been reported for the dimethyl derivative.[6] Calculations for the neutral histamine, the mono- and dication include semiempirical quantum chemical methods,[4,12a-c] ab initio calculations using the STO-3G,[12d] STO-4G,[12e] and the 6-31G[12g] basis sets, and consideration of the molecular electrostatic potentials.[12b,d,f] The activation mechanism of the histamine H_2 receptor was studied by Weinstein et al.[13] using split valence ab initio basis sets and 6-31G single point energy calculations. Common in these studies is that no diffuse functions or electron correlation, which are essential in consideration of hydrogen bonding,[14] was used. Furthermore only some selected conformations were investigated and without consideration of solvent effects but predicting relative pK_a values for histamine and its methyl derivatives.[15] There exist conformational equilibria for other β-substituted ethylamine systems, e.g., dopamine, that show large sensitivity to solvation and the pH of the medium. Theoretical calculations are capable of pointing out this effect.[16]

The most comprehensive computational study for the isolated neutral histamine molecule was reported by Vogelsanger et al.[17] Six trans and 14 gauche conformers were compared after total geometry optimization using the 3-21G basis set. Our aim in this article is to report a theoretical investigation of the conformational equilibrium for histamine and (αR,βS)-α,β-dimethylhistamine (from here on "dimethylhistamine") in aqueous solution, obtained by combining ab initio quantum chemical calculations and Monte Carlo simulations. Due to the foreseeable large computational effort only the most stable conformations have been chosen also in the present study. Starting from the results of Vogelsanger et al.,[17] the lowest energy gauche and trans conformers at the two tautomeric structures of the imidazole ring were selected for further investigations both in the neutral and the monocationic form. The dicationic form is not considered in this paper due to the low population of this species predicted to be present under physiological conditions. After total geometry optimization in the gas phase, the free energies of the tautomers/conformers were determined. Relative free energies of hydration were obtained by using the statistical perturbation method within Monte Carlo simulations of the dilute aqueous solutions. The results highlight the different energy contributions regulating the conformational equilibrium in aqueous solution both for histamine and its dimethyl derivative. Analysis of the solution structure in the first hydration shell of the polar sites may provide a useful addition to understanding the dehydration process that may take place before binding to histamine receptors.

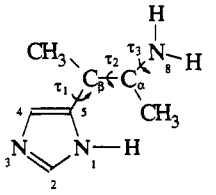

Figure 1. Atom numbering and definition of the axes with torsion angles of τ1, τ2, and τ3.

Methods and Calculations

The numbering system used throughout this paper is shown in Figure 1. [The IUPAC IUB Commision on Biochemical Nomenclature introduced the system for histidine,[18a] and was applied for histamine,[18b] that the ring nitrogen nearer and farther from the side chain be designated by symbols N^{π} and N^{τ}, respectively. In the present study, in order to make the comparison with results in refs 12, 13, and 17 easier, the following numbering is used: N1 is the ring nitrogen atom next to the side chain, N3 is the ring nitrogen farther from it, corresponding to N^{π} and N^{τ}, respectively. Thus C5 is the ring carbon atom bearing the side chain: C_β–C_α–N8. N8 is the nitrogen atom in the amine group. Torsional angles are defined: τ1 = N1–C5–C_β–C_α, τ2 = C5–C_β–C_α–N8, and τ3's = C_β–C_α–N8–H. Conformations will be designated by gauche (g) and trans (t) when τ2 is about ±60° and 180°, respectively. Tautomers will be designated by symbols 1H and 3H added to the symbol of the conformation of g or t, depending on the location of the ring hydrogen on N1 or N3, respectively. The protonated structures will be designated by the symbol + added to the name. Thus g1H refers to the gauche, neutral structure bearing a hydrogen at N1, t3H+ refers to a trans conformation where the ring hydrogen is located at N3 and the monocation is protonated at N8. Dimethylhistamine molecules are indicated by the letters dm preceding the other symbols, e.g., dmg1H, dmt3H+.] Ab initio total geometry optimizations have been carried out for the following systems in the gas phase: histamine g1H, g3H, t1H, t3H, g1H+, g3H+, t3H+, and t1H+ and dimethylhistamine dmg1H, dmg3H, dmt1H, dmt3H, dmg3H+, and dmt3H+. The calculations were done on a Stardent Titan 3040 computer at the University of Toledo and on a Cray Y-MP8 at the Ohio Supercomputer Center utilizing the Gaussian 90 package.[19] Starting from the geometries obtained by Vogelsanger et al.[17] and removing the restriction for the coplanarity of the ring atoms applied in that study, the structures were optimized using the 3-21G basis set and then reoptimized using the 6-31G* basis set.[20] All histamine

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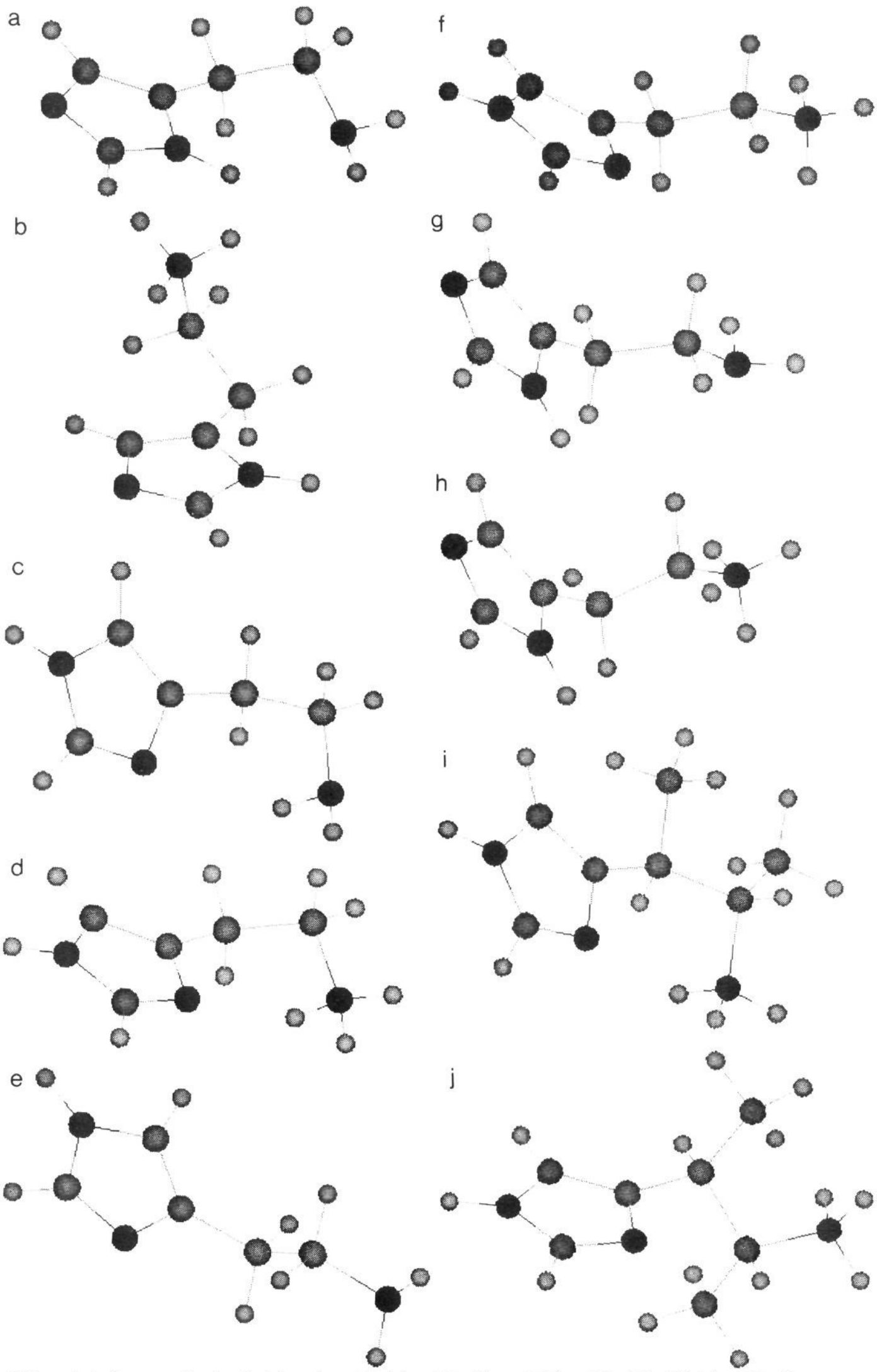

Figure 2. HF/6-31G* optimized geometries for the histamine g1H (a), g1H+ (b), g3H (c), g3H+ (d), t3H (e), t3H+ (f), t1H (g), and t1H+ (h) structures and for the (αR,βS)-α,β-dimethylhistamine monocation in the dmg3H+ (i) and dmt3H+ (j) conformations. For the structure name convention, see the text.

structures are of C_1 symmetry, thus every conformation exists as a pair of optical antipodes with equal energies. Data in Table 1 and structures in Figure 2 are given for the histamine conformers with negative $\tau 1$ values. For the dimethyl derivative where the specific (αR,βS) isomer was studied conformers with negative and positive $\tau 1$ values are of different energy. Thus for the dimethyl derivative two sets of conformers have been calculated. Setting $\tau 1$ and the corresponding $\tau 2$ values to those optimized for histamine, the **a** and **b** sets for dimethylhistamine were obtained starting from the negative and positive $\tau 1$ values, respectively.

The more important geometric parameters optimized at the 6-31G* level are summarized in Table 1. Normal frequency analyses proved all histamine structures were local energy minima. For the dimethyl derivative normal frequency analyses were carried out only for the protonated gauche and trans forms. The N–H stretching frequencies for the free bonds and for those in intramolecular hydrogen bonds are compared in Table 2. Using normal frequencies, relative zero point energies and thermal corrections for the enthalpy, entropy, and free energy were calculated in the rigid rotor-harmonic oscillator approximation.[21]

Table 1. HF/6-31G* Optimized Geometric Parameters for Histamine and Dimethylhistamine

	g1H	g3H	t3H	t1H	g1H+	g3H+	t3H+	t1H+
				Histamine				
τ1	−46.6	−63.8	−66.9	−72.6	−151.6	−41.0	−50.5	−86.7
τ2	67.7	68.1	177.5	177.7	57.0	62.8	170.5	−177.9
τ3	−171.6	−53.6	−61.3	−174.3	−52.8	−50.0	−59.9	−60.2
	70.7	62.9	57.4	67.5	66.0	68.0	59.9	59.6
					−173.0	−171.0	−179.7	179.5
N_{ring}–H	0.996	0.993	0.993	0.993	0.996	0.996	0.995	0.995
N_{am}–H	1.002	1.002	1.002	1.002	1.011	1.009	1.011	1.011
	1.003	1.003	1.002	1.002	1.011	1.009	1.011	1.011
					1.015	1.031	1.011	1.011
N···H	2.314	2.458			3.474	1.855		
N···N	2.947	3.163			4.000	2.718		
N–H···N	120.5	127.0			114.3	138.9		
$C_α$–$C_β$	1.531	1.540	1.536	1.532	1.525	1.528	1.524	1.529
N_{am}–$C_α$	1.462	1.452	1.454	1.454	1.512	1.508	1.520	1.521
				(αR,βS)-α,β-Dimethylhistamine[a]				
τ1	−48.4	−61.0	60.7	65.4		−44.3	51.7	
τ2	67.6	70.1	−177.1	178.2		63.8	−170.9	
τ3	−175.3	−55.9	63.5	172.7		−51.5	−59.3	
	67.2	60.5	−56.2	−69.8		66.2	61.2	
						−172.9	−178.8	
N_{ring}–H	0.996	0.993	0.993	0.993		0.996	0.995	
N_{am}–H	1.003	1.003	1.000	1.002		1.009	1.009	
	1.003	1.003	1.003	1.004		1.009	1.010	
						1.029	1.011	
N···H	2.269	2.390				1.859		
N···N	2.910	3.107				2.720		
N–H···N	121.0	127.7				138.8		
$C_α$–$C_β$	1.547	1.557	1.552	1.547		1.544	1.540	
N_{am}–$C_α$	1.467	1.456	1.459	1.460		1.520	1.533	

[a] Geometries of the more stable gauche and trans structures in Table 3.

Table 2. Calculated N–H Stretching Frequencies in the Gas Phase[a]

	N_{ring}–H	N_{am}–H
	Histamine	
g1H	3879	3804, 3721
g3H	3921	3809, 3724
t3H	3922	3802, 3719
t1H	3910	3809, 3726
g1H+	3889	3737, 3591, 3696
g3H+	3892	3755, 3301, 3687
t3H+	3900	3736, 3733, 3630
t1H+	3898	3730, 3727, 3625
	(αR,βS)-α,β-Dimethylhistamine	
dmg3H+	3894	3757, 3331, 3690
dmt3H+	3900	3752, 3740, 3638
imidazole[b]	3921 (*a'*)	
methylamine[c]		3809 (*a''*), 3728 (*a'*)
methylammonium cation[c]		3724 (*e*), 3624 (a_1)

[a] Unscaled HF/6-31G* frequencies in cm^{-1}. [b] Reference 22. [c] This work.

A frequency scaling factor of 0.9[20] was used when calculating zero point energies (ZPE) but not for thermal corrections to the relative free energy.[1,2,22] Single point calculations were carried out for the histamine structures at HF/6-311++G**, MP2/6-31G*, and MP2/6-311++G** levels using the HF/6-31G* optimized geometries. Electron correlation beyond the MP2 level has proven important to estimate the 2-hydroxypyridine/2-pyridone tautomeric equilibrium.[23a] Thus, QCISD calculations (quadratic configuration-interaction method with singles and doubles)[23b] providing also the MP4SDQ energies were carried out in the 6-31G* basis. MP2/6-31G*//HF/6-31G* single point calculations were done for the dimethylhistamine tautomers/conformers. Energy values are summarized in Table 3. The basis set effect on the histamine t3H+ – g3H+ energy difference were studied by using some other basis sets at the HF and MP2 levels (Table 4).

Monte Carlo simulations of histamine to obtain relative free energies in aqueous solution[24] using the statistical perturbation method[25] were applied for the neutral g1H–g3H, g3H–t3H, t3H–t1H and protonated gH3+–t3H+, g3H+–g1H+, t3H+–t1H+ pairs. The dmg3H+–dmt3H+ conformational change was followed for the dimethyl derivative. The simulations were carried out, using Jorgensen's BOSS 3.1 program[26] ported by us to both a Stardent Titan 3040 and a DEC Alpha OSF/1 computer, for isothermal-isobaric (NPT) ensembles at T = 298 and P = 1 atm. Details of such calculations have been described by Jorgensen et al.[27] The solute molecule was placed in the center of a box containing 258 water molecules. TIP4P water model[28] and models with 11–14 atomic centers were applied to the solutes with united CH_x atoms (x = 1–3). Periodic boundary conditions and preferential sampling,[29] proportional to $1/(R^2 + c)$, were applied where c was set equal to 120. R is the distance between the water molecule and the midpoint of the C5–$C_β$ bond. The 12-6-1 interaction potential was used (for parameterization see below) taking cut-off radii of 9.75 Å for the solute–solvent and of 8.5 Å for the solvent–solvent interactions. Generation of new configurations upon change of the solute position was attempted after every 50 steps. Volume changes were tried after 1000 steps. Statistical averaging was taken for 10^5 configurations in separate runs. In calculating free energies the solutes were transformed into each other using a linear coupling parameter, λ. Thus, when transforming solute a to solute b the geometric and simulation parameters, P(a) and P(b) changed according to

$$P(\lambda) = (1 - \lambda)P(a) + \lambda P(b)$$

where λ = 0 for the a and λ = 1 for the b structure. Energy values were obtained in backward and forward simulations allowing Δλ = 0.05–0.08 in the steps of transformation. Configurations (3×10^6) were taken for each of the equilibration and averaging phases in every step. Solution structure characterizations are based on data obtained in simulations with consideration of at least 4.5×10^6 configurations in each phase.

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Table 3. Relative Energies and Free Energies in the Gas Phase[a]

	g1H	g3H	t3H	t1H	g3H+	t3H+	g1H+	t1H+
				Histamine				
3-21G//3-21G	0.00	3.51	5.03	5.62	0.00	18.84	26.84	31.68
6-31G*//6-31G*	0.00	2.32	3.05	3.38	0.00	12.49	19.66	23.97
6-311++G**	0.00	2.02	2.12	2.66	0.00	11.75	19.37	23.43
MP2/6-31G*	0.00	2.58	4.35	5.28	0.00	15.31	21.47	27.32
MP4SDQ/6-31G*	0.00	2.44	4.02	4.84	0.00	14.69	21.06	26.54
QCISD/6-31G*	0.00	2.42	3.99	4.80	0.00	14.66	21.04	26.45
MP2/6-311++G**	0.00	2.53	3.40	4.28	0.00	14.20	20.41	26.14
0.9*ZPE[b]	0.00	–0.34	–0.59	–0.49	0.00	–0.40	–0.33	–0.67
$H - TS$[b]	0.00	–0.39	–0.53	–0.59	0.00	–0.77	–0.45	–0.99
G_{gas}[c]	0.00	1.80	2.28	3.20	0.00	13.03	19.63	24.28
				(αR,βS)-α,β-Dimethylhistamine				
3-21G//3-21G[d]	0.00	3.61	3.80	4.33	0.00	16.43		
6-31G*//6-31G* **a**	0.00	2.69	4.49	4.42	0.00	13.96		
6-31G*//6-31G* **b**	0.26	2.22	3.10	3.53	0.10	11.22		
MP2/6-31G* **a**	0.00	3.04	5.28	5.60	0.00	16.03		
MP2/6-31G* **b**	0.73	3.12	4.23	4.94	0.44	13.43		
0.9*ZPE[e]					0.00	–0.46		
$H - TS$[e]					0.00	–0.80		
G_{gas}[e]					0.00	12.17		

[a] Relative energies in kcal/mol. If not indicated, HF/6-31G* optimized geometry was used. [b] Relative zero point energy corrections (ZPE), thermal corrections to enthalpy (H) and entropy (S) at $T = 298$ were calculated using HF/6-31G* normal frequencies. [c] G_{gas} values are the relative free energies in the gas phase calculated using the highest level MP2 relative energies. [d] Energy values for the a set. [e] Data refer to the conformers dmg3H+ (**a**) and dmt3H+ (**b**).

Table 4. Basis Set Effect on the Histamine t3H+ – g3H+ energy[a]

	E(t3H+) – E(g3H+)	
	HF	MP2
6-31G*	12.49	15.31
6-31G**	12.74	15.48
6-31+G**	11.85	14.13
6-31++G**	11.82	14.14
6-311++G**	11.75	14.20

[a] Relative single point energies in kcal/mol. Geometry optimized at HF/6-31G* level.

For the molecular geometries of the solutes the gas-phase optimized structures were taken. Intramolecular hydrogen bond formations in the gauche conformations result in charge distributions different from those in the trans forms and should be considered to find relevant parameters for solution simulations. Conformation dependent charges were necessary for obtaining quantitative accuracy in the conformational energy for *N*-methylacetamide[30a] or acetic acid[30b] in aqueous solution. To explore its significance for the present system two sets of parameters were applied for the pair of monocations g3H+ and t3H+. In set1 the Jorgensen OPLS parameters[31] were applied in the 12-6-1 interaction potential without distinction for the corresponding charges in the gauche and trans conformations. Parameters for the imidazole part were the same as used for simulations in water.[22] CH_x parameters for the side chain were chosen from those available in the BOSS 3.1 program, and values for the protonated amine group were taken from Jorgensen and Gao.[27c] For set2 we used a scaling procedure as follows.[2] The Mulliken charges reflect the polarization of charges upon intramolecular hydrogen bond formation for the gas-phase histamine conformers. Thus HF/6-31G*//HF/6-31G* charges were calculated for the free imidazole, methylamine, and methylammonium cation, as building block molecules for neutral and protonated histamine at their optimized geometry, and the charges for the histamine conformers relative to their imidazole N(H) atom were divided with the corresponding values in the building blocks to get atomic scaling factors. The nonunit scaling factors were considered due to polarization effect upon hydrogen bond formation. The Mulliken charges are, however, much larger in absolute value than those used as OPLS charges parameterized against experimental measurables. To maintain the near OPLS charge magnitude but incorporating the polarization effect, the OPLS charges relative to the imidazole N(H) value in set1 were multiplied by the above scaling factors to obtain the new relative charge parameters for the solutes in set2. In set1 and set2 the simulation charge parameters for (H)N3 nitrogen atom (not involved in intramolecular hydrogen bond in the monocation) were identical. To maintain the unit positive charge for the total histamine monocation the atomic charges for C_β were adjusted accordingly. For the neutral species generally the OPLS parameters were taken, while the parameters for the NH_2 group were taken from simulations giving good enthalpy and relative free energy of solvation.[32] The applied sets are given in Table 5. The values for n ($E \leq E_{min}$) in Table 8 represent the number of solvent molecules with solute–solvent interaction energy no larger than E_{min}, the first minimum in the energy pair distribution function. Numbers in the second column were calculated when integrating up to E_{ul}, the value assumed as the upper limit for the solute–solvent intermolecular hydrogen bond energy in the distribution curve. The total solute–solvent interaction energy is given by E_{SX}.

Table 5. Parameters of the 12-6-1 Potential Function for Solution Simulations

	q, au					
		protonated				
	neutral	set1[a]	set2g[b]	set2t[b]	σ, Å	ϵ, kcal/mol
N	–0.490	–0.490	–0.560	–0.513	3.250	0.170
C2	0.410	0.410	0.499	0.466	3.750	0.145
N(H)	–0.570	–0.570	–0.570	–0.570	3.250	0.170
C4	0.130	0.130	0.172	0.165	3.750	0.145
C5	0.100	0.100	0.152	0.113	3.750	0.145
H(N)	0.420	0.420	0.449	0.443	0.0	0.0
C_β[c]	0.000	0.000	0.026	0.016	3.905	0.118
C_α[c]	0.250	0.310	0.203	0.234	3.905	0.118
N_{am}[d]	–1.050	–0.300	–0.352	–0.326	3.250	0.170
H_{am}	0.400	0.330	0.356	0.321	0.0	0.0
	0.400	0.330	0.310	0.323	0.0	0.0
		0.330	0.315	0.328	0.0	0.0
Me_β		0.000			3.910	0.160
Me_α		0.000			3.910	0.160

[a] Parameters used for g3H+ and t3H+. For g1H+ and t1H+ parameters for C4 and C5 are interchanged. [b] Set2g and set2t were used for g3H+ and t3H+, respectively. [c] σ and ϵ values are 3.850 and 0.080, respectively for the simulations with the dimethylhistamine solute, considering these atoms as tertiary carbons. [d] σ is 3.300 in simulations with neutral histamine solutes.

Results and Discussion

Geometries. Some important geometric parameters are summarized in Table 1, obtained from HF/6-31G* total geometry

(30) (a) Jorgensen W. L., Gao, J. *J. Am. Chem. Soc.* **1988**, *110*, 4212. (b) Pranata, J. *J. Comput. Chem.* **1993**, *14*, 685.
(31) (a) Jorgensen W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.
(32) (a) Dunn III, W. J.; Nagy, P. I.; Collantes, E. R. *J. Am. Chem. Soc.* **1991**, *113*, 7898. (b) Dunn III, W. J.; Nagy, P. I. *J. Phys. Chem.* **1990**, *94*, 2099.

optimizations (Figure 2). The bond lengths and angles for the imidazole ring (not included in Table 1) are basically unchanged as compared to the HF/6-31G* optimized geometry determined previously.[22] The deviations from that structure in histamine and dimethylhistamine are at most some thousandths of an Å for bond lengths and some tenths of a degree for bond angles. Thus neither tautomerism and conformational changes nor even protonation at the side chain affect remarkably the gas-phase ring geometry.

The optimized values of the $\tau 2$ and $\tau 3$ torsion angles are only moderately sensitive to hydrogen bond formation. Values differ by no more than 10° from ideal angles of ±60° and 180° for the gauche and trans arrangements. In contrast, changes of the $\tau 1$ values for histamine amount to 26° for the neutral and 46° for the protonated species (excluding the g1H+ structure, vide infra), indicating that the torsion for the imidazole ring is sensitive to the global conformation. The intramolecular hydrogen bonds N1–H···N8 in g1H and the N1···H-N8 in g3H differ considerably and are in accord with the results of Alagona et al.[33] who pointed out that the sp^3 nitrogen forms shorter hydrogen bonds when acting as proton acceptor rather than donor. In fact, the N···N and H···N distances in Table 1 are shorter for g1H than for g3H. Also a hydrogen bond was found in g3H+ with an N1···H distance of 1.86 Å. The $\tau 1$ torsion angles correlate with the intramolecular hydrogen bond formation: $\tau 1$ is in the range of –41° to –47° for gauche conformers with shorter and thus stronger hydrogen bonds (g1H and g3H+), while the torsion angle is larger, –51° to –87°, for g3H with a weaker intramolecular hydrogen bond and for the trans conformers without such bonds. Figure 2a,b shows that the most remarkable change in the conformation between the neutral and monocationic forms emerges upon the protonation of g1H to form g1H+. The large repulsion between the H(N1) and H(N8) atoms after adding a proton to the NH_2 group in g1H is removed by a considerable torsion about the C_5–C_α bond. $\tau 1$ changes from –41° in g1H to –152° in g1H+ (Table 1). As a result the NH_3^+ group is at nearly equal distances from N1 and N3 (3.47 Å *versus* 3.53 Å). This long N–N distance prevents the formation of an intramolecular hydrogen bond but allows the protonated group to interact with the π electron cloud of the imidazole ring (Figure 2b).

For the dimethylhistamine (Figure 2i,j) the geometric data of the most stable conformers are shown in Table 1. Based on the MP2/6-31G* relative energy values in Table 3, parameters for the gauche and trans structures refer to the **a** and **b** sets, respectively, corresponding to –/+ and +/– sign combinations of the $\tau 1/\tau 2$ torsion angles. In studying the effect of the two methyl groups on the optimized geometries no steric repulsion was found in any conformations. Absolute values of the $\tau 1$, $\tau 2$, and $\tau 3$ angles are similar to those of the histamine conformers and the hydrogen bond parameters for dmg1H, dmg3H, and dmg3H+ are close to those of g1H, g3H, and g3H+, respectively. Thus the dimethylation in the (αR,βS)-α,β-dimethylhistamine does not affect considerably the above geometric parameters in the most stable conformers. Considering, however, the opposite sign for the $\tau 1$ angle, larger deviations were found.

The gauche conformers in the **b** sets (not indicated) have similar absolute values for $\tau 1$ and $\tau 2$ as do the **a** set structures in Table 1. For the trans structures, however, the $\tau 1$ values for the higher energy dmt3H, dmt1H, and dmt3H+ trans conformes (**a** set structures in Table 3) are –101°, –100°, and –78° *versus* 61°, 65°, and 52° in Table 1, respectively. Comparing the $\tau 1$ absolute values, the increase in energy is attributed to the larger torsion about the C5–C_β bond and reflects the different effect of the two methyl groups on the trans conformer geometry with positive and negative signs for $\tau 1$. Only a minor effect was found with the gauche structures where the hydrogen bond formation maintains the similar torsion about C5–C_β.

N_{ring}–H bond lengths in Table 1 are 0.993–0.996 Å, equal or slightly longer than the value of 0.993 Å for imidazole. In the protonated structures the ring hydrogen is not involved in hydrogen bond and the longer N–H bond is attributed to the electrostatic repulsion between the protonated amine group and the ring hydrogen. The effect of the hydrogen bond formation is much more pronounced for the NH_3^+ group. The N–H distance of 1.012 Å in the methylammonium cation changes only a little for the g1H+, t3H+, and t1H+ ions in Table 1. There is, however, one N–H bond in both g3H+ and dmg3H+ which is the proton donor in the intramolecular hydrogen bond and is stretched by almost 0.02 Å to 1.029–1.031 Å. Protonation decreases the C_β–C_α bond length generally by 0.012 Å as compared to the corresponding neutral species. The effect is considerably larger for the C_α–N8 bonds: protonation increases these bonds by 0.05–0.07 Å. At the same time the N···N distances become shorter in the gauche 3H conformers by 0.39–0.45 Å as compared to the neutral species.

Vibrational Frequencies. Unscaled HF/6-31G* N–H stretching frequencies for histamine, the dimethylhistamine, and for the building block molecules imidazole, methylamine, and methylammonium cation are compared in Table 2. The calculated N_{ring}–H stretching frequency for the g3H and t3H tautomers of histamine is the same as in the unsubstituted imidazole, 3921 cm^{-1}.[22] The N_{ring}–H bond does not participate in intramolecular hydrogen bonding in these conformers and our calculation predicts that the N_{ring}–H frequency is not effected by the β-substitution of the imidazole ring. The frequency for t1H is 3910 cm^{-1}, lower than that for imidazole, and reflects the effect of the α-substitution of the ring. The frequency is remarkably decreased for g1H where the N_{ring}–H bond is involved in the N1–H···N8 hydrogen bond. Lowering of the stretching frequencies upon formation of hydrogen bonds was also found in previous calculations[1,2,22] and is well-known experimentally.[34] The N_{ring}–H frequencies for the monocations are lower by 21–32 cm^{-1} than the value for imidazole. The decrease in the frequencies occurs even if the N–H bonds are not involved in intramolecular hydrogen bonds and is in line with the larger N–H distances found for the monocations (Table 1).

Two ranges of the N_{am}–H stretching frequencies were found for the neutral histamine structures: 3802–3809 cm^{-1} and 3719–3726 cm^{-1}. They are similar to those obtained for methylamine with C_s symmetry: 3809 cm^{-1} (a') and 3728 cm^{-1} (a'). The calculated values do not differ as much for the N_{am}–H frequencies as for the N_{ring}–H values. They are almost independent of whether the N_{am}–H bond is involved in a hydrogen bond or whether the NH_2 group is proton donor or acceptor in that bond.

N_{am}–H stretching frequencies are similar for g3H+ and dmg3H+ as well as for t3H+, t1H+, and dmt3H+. Intermediate values were calculated for g1H+ without an intramolecular hydrogen bond but with possible interaction of the NH_3^+ group and the π electrons of the ring. Contrary to the neutral species, the values are much different for the trans and gauche conformations. The calculated values for the methylammonium cation with C_{3v} symmetry are 3724 (e) and 3624 cm^{-1} (a_1). The symmetric stretching frequencies are little changed for the trans conformers (3625–3638 cm^{-1}) but are considerably increased for the gauche forms (3687, 3690 cm^{-1}). The degenerate e mode of the methylammonium cation is split for the gauche 3H forms by 426–454 cm^{-1} but only by 146 cm^{-1} for g1H+. The N_{am}–H stretching frequencies for the N–H bonds, donor in intramolecular hydrogen bonds, are at 3301–3331 cm^{-1}. The split is 3–12 cm^{-1} for the trans conformers, and the frequencies are slightly increased into the range of 3727–3752 cm^{-1}.

Gas-Phase Energies. Relative energies obtained using different basis sets are shown in Table 3. All the calculations agree in the predicted order of the relative energies. In contrast, Topiol et

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(34) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons: New York, 1981.

al.[12d] found the neutral t1H form more stable than t3H by 0.99 kcal/mol at the HF/STO-3G level, opposite to the results at any levels in Table 3. The relative energy for the protonated species was calculated close to the present values.

Calculations using the 3-21G basis set give the largest energy separations, while those based on the HF/6-31G* optimized geometries with larger basis sets give more uniform relative energy values. Using the 6-31G* basis set the tautomers/conformers are more separated in energy than when using the 6-311++G** basis set in the single point energy calculations. Results at MP2 level indicate larger energy separations than at the HF level. These general statements apply both to histamine and the dimethylhistamine and to neutral and protonated species.

The lowest energy neutral species corresponds to the g1H arrangement. Formations of the N1–H···N8 intramolecular hydrogen bond is considered the most important factor stabilizing this structure. The next stable structure, g3H, is higher in energy for histamine by 2.0–2.3 kcal/mol at the HF level using basis sets larger than 3-21G. Calculations considering electron correlation give somewhat larger values. The close agreement of the MP2, MP4SDQ, and QCISD relative energies suggests a converged relative energy at about 2.5 kcal/mol. The N1···H–N8 hydrogen bond is expected to be weaker than the N1–H···N8 bond, as was discussed based on the geometric data in the previous section. This weakening of the hydrogen bond is considered as the main reason for the destabilization relative to g1H. The t3H is the more stable trans conformer with relative energy of 2.1–4.4 kcal/mol, while the relative energy of t1H is 2.7–5.3 kcal/mol. QCISD/6-31G* relative energies are smaller than the MP2/6-31G* values for these conformers by 0.4–0.5 kcal/mol but are almost equal with the MP4SDQ conformer energies. Thus calculations beyond the MP4 level may lead to minor effects for the relative energies. Considering the finding of Del Bene[14] that the MP2 and MP4SDTQ binding energies for neutral and protonated hydrogen bound complexes differ by 0.1–0.2 and 0.2–0.4 kcal/mol, respectively, using basis sets augmented with diffuse functions, the relative energies at the MP2/6-311++G** level may be considered reliable within a few tenths of a kcal/mol.

Monocationic forms without intramolecular hydrogen bonds are higher in energy by 11–24 kcal/mol than the most stable g3H+ structure at the HF/6-31G* and 6-311++G** levels; the relative energies are in the range 14–28 kcal/mol considering electron correlation. The relative energies of the t3H+, g1H+, and t1H+ conformers decrease by 0.4–0.8 kcal/mol going from the MP2 to MP4SDQ/6-31G* level but remain stable within 0.1 kcal/mol at the QCISD/6-31G* level. Using the same argument as for the neutral species above the MP2/6-311++G** relative energies for the protonated histamine conformers are considered reliable within a few tenths of a kcal/mol. The relative stability of 19.4–21.5 kcal/mol for histamine g1H+ is explained by the balance of the repulsive and attractive interactions of the NH_3^+ group with the N1–H and N3 atoms, respectively. The t1H+ isomer is higher in energy than g3H+ by 23.4–27.3 kcal/mol and thus is higher in energy than t3H+ by 11.5–12.0 kcal/mol. Despite the conformational similarity of t3H+ and t1H+ for histamine, the N8···H(N) distance between the cationic head NH_3^+ group and the ring H atom connecting to a nitrogen is 6.9 Å for t3H+ and 4.8 Å for t1H+. Calculating the electrostatic interaction energies between the NH_3^+ group and the polar parts of the imidazole ring (N–H and N) using the 6-31G* atomic charges, the interaction energy is more negative for the t3H+ tautomer by 5 kcal/mol, accounting for nearly the half of the relative HF energy. The large contribution suggests an electrostatic stabilization of t3H+ having the cationic head more apart from the positive H(N) atom in this tautomer as compared to t1H+.

Due to computer time limitations the highest level results provided for the dimethyl derivatives are MP2/6-31G* relative energies. These are generally lower for the gauche conformers in the **a** set and for the trans forms in the **b** set. The sequence of the most stable tautomers/conformers is identical to that for histamine, both at the HF/6-31G* and MP2/6-31G* levels. The corresponding relative energies of the histamine and dimethylhistamine structures may differ by some tenths of a kcal/mol for the neutral species. The dmt3H+ cation is higher in energy than dmg3H+ by 11–14 kcal/mol for the dimethyl derivative in set **b**. Comparable results for histamine and dimethylhistamine at the HF and MP2 levels with the 6-31G* set show small differences for the neutral forms, but the relative energy is lower by 1–2 kcal/mol for the protonated dimethyl derivative.

The wide relative energy range for the histamine trans conformers are due to the different stabilizations at the HF and MP2 levels. The stabilization of structures with compared to without intramolecular hydrogen bonding is larger at the MP2 and QCISD/6-31G* levels than in the HF/6-31G* calculations, as was found both for 1,2-ethanediol[1] and 2-OH benzoic acid.[2] In the present case the tendency of the larger stabilization by MP2 was found even using the larger 6-311++G** basis set.

Relative zero point energies (ZPE) calculated from normal frequencies determined at the HF/6-31G* level and scaled by a factor of 0.9 are 0.3–0.7 kcal/mol. The thermal corrections to the relative gas-phase free energy, $\Delta H - T\Delta S$; are 0.4–0.6 kcal/mol for the neutral and 0.5–1.0 kcal/mol for the protonated species. The relative ZPE and thermal correction values for the neutral molecules are similar to those found in previous calculations for internally hydrogen bonded systems.[1,2] The relative values for the monocations are larger than those for the neutral species by up to 0.4 kcal/mol. The consistently negative signs for the relative ZPE values mean that the zero point energy is larger in g1H than in the other neutral structures. Also ZPEs for g3H+ and dmg3H+ are larger than those for the corresponding trans conformers despite the lower stretching frequencies for vibrations involved in hydrogen bond in the reference arrangements. Thus the larger ZPE for the reference conformations is possible only due to larger contributions from other lower energy stretching, bending, and torsional vibrations. The thermal corrections are larger for the different conformations than for the corresponding reference systems by at least 0.1 kcal/mol for the enthalpy and 0.5 kcal/mol for the entropy, giving positive ΔH and ΔS terms. The $\Delta H - T\Delta S$ differences are dominated by the entropy terms thus leading to a negative value.

The basis set effect on the relative energy was studied for the t3H+ – g3H+ conformer pair of histamine (Table 4). Single point calculations at the HF/6-31G* optimized geometries show a relative energy decrease of 0.7–1.1 kcal/mol upon basis set extension. A significant change both at the HF and MP2 levels was found when adding one set of diffuse functions, i.e., using the 6-31+G** basis set.[20] Addition of a second set of diffuse functions, 6-31++G**, or use of a triple split valence set, 6-311++G**, resulted in no further improvement, in agreement with Del Bene's results on calculated interaction energies for hydrogen bonded dimers.[14] Our results also indicate that a convergence for the relative energies of the most important conformers has been achieved within our computational limit for this size of a molecule.

The relative free energies in the gas phase were calculated based on the MP2/6-311++G**//HF/6-31G values for histamine (Table 3). Due to the frequency-related corrections the relative free energies of the all tautomers/conformers are considerably decreased in comparison with the corresponding energy values. Though the relative free energy order for the structural isomers has not changed, consideration of the frequency dependent terms are important for systems where the relative energies are 2–5 kcal/mol. Such calculations, even with the rather small effect for the ionic systems in the gas phase, become more important if the relative free energies in aqueous solution are to be determined as follows in the next section.

Equilibrium in Solution. Solvation free energy changes upon tautomerization or conformational changes are comparable to the relative free energy values in the gas phase (Table 6). The tautomerizations from g1H to g3H and from t1H to t3H for

Table 6. Relative Solvation Free Energies[a]

	forward			backward		
	ΔG	ΔH	$T\Delta S$	ΔG	ΔH	$T\Delta S$
			Histamine			
g1H to g3H	−3.43(0.25)	3.0(3.5)	6.5(3.5)	4.06(0.29)	8.0(3.1)	3.9(3.1)
g3H to t3H	−0.70(0.25)	−28.7(4.5)	−28.0(4.5)	2.54(0.27)	11.8(4.5)	9.3(4.6)
t3H to t1H	0.63(0.26)	13.5(3.4)	12.9(3.4)	−1.14(0.27)	−0.4(3.3)	0.8(3.2)
g3H+ to t3H+						
set1	−10.62(0.28)	4.7(4.2)	15.3(4.2)	11.31(0.26)	−2.3(4.2)	−13.6(4.1)
set2	−10.62(0.26)	−27.8(4.2)	−17.2(4.2)	11.43(0.28)	22.6(4.5)	11.1(4.6)
t3H+ to t1H+	−12.97(0.19)	−12.5(3.0)	0.5(3.0)	13.30(0.19)	8.1(3.2)	−5.2(3.1)
g3H+ to g1H+	−10.02(0.57)	−51.6(9.1)	−41.5(8.9)	14.50(0.55)	−9.1(9.0)	−23.6(8.9)
			(αR,βS)-α,β-Dimethylhistamine			
dmg3H+ to dmt3H+	−7.37(0.62)	13.7(8.5)	21.1(8.6)	11.18(0.68)	−47.3(11.2)	−58.4(10.9)

[a] Relative solvation free energies (ΔG), enthalpies (ΔH), and entropies ($T\Delta S$) in kcal/mol. Values in parentheses are $\pm\sigma$. For set1 and set2, see Table 5.

Table 7. Total Relative Free Energies in Solution[a]

Histamine[b]				
	g1H	g3H	t3H	t1H
G_{gas}	0.00	1.80	2.28	3.20
G_{sol}	0.00	−3.74(0.19)	−5.36(0.26)	−4.48(0.32)
G_{tot}	0.00	−1.94(0.19)	−3.08(0.26)	−1.28(0.32)
fraction in soln	1%	12%	83%	4%
	g3H+	t3H+	t1H+	g1H+
G_{gas}	0.00	13.03	24.48	19.63
G_{sol}	0.00	−10.96(0.19)	−24.10(0.23)	−12.2(0.4)
G_{tot}	0.00	2.07(0.19)[b] (3.18(0.19)[c])	0.38(0.23)	7.4(0.4)
fraction in soln	64%	2%	34%	
(αR,βS)-α,β-Dimethylhistamine[c]				
		g3H+	t3H+	
G_{gas}		0.00	12.17[c]	
G_{solv}		0.00	−9.28(0.42)	
G_{tot}		0.00	2.89(0.42)[c]	

[a] Values in kcal/mol. G_{sol} values are averages from Table 6. [b] Calculated using MP2/6-311++G** energies. [c] Calculated using MP2/6-31G* energies.

Table 8. Solute–Solvent Interaction Energies[a]

	$n\ (E \le E_{min})$	$n\ (E \le E_{ul})$	E_{SX}[b]	DM_{gas}	DM_{sol}
		Histamine			
g1H	2.3(−3.50)	4.7(−2.5)	−46	5.65	5.10
g3H	2.7(−3.50)	4.7(−2.5)	−51	4.73	4.92
t3H	1.9(−4.50)	5.0(−2.5)	−53	4.45	4.75
t1H	2.7(−3.75)	5.2(−2.5)	−52	3.46	3.06
g3H+	2.5(−10.0)	3.8(−8.0)	−133		
set2[c]		3.8(−8.0)	−126		
t3H+	3.6(−10.0)	4.6(−8.0)	−155		
set2[c]		4.7(−8.0)	−146		
t1H+	2.7(−12.0)	6.1(−8.0)	−186		
g1H+	2.8(−11.0)	5.0(−8.0)	−169		
		(αR,βS)-α,β-Dimethylhistamine			
dmg3H+	2.4(−10.0)	3.6(−8.0)	−133		
dmt3H+	3.6(−10.0)	4.9(−8.0)	−158		
imidazole[b]	0.4(−5.50)	3.0(−2.5)	−27	3.86	3.90
CH_3NH_2[c]	1.2(−4.00)	2.1(−2.5)	−17	1.53	1.87
$CH_3NH_3^+$ [c]	3.5(−10.0)		−136		

[a] For definition of the terms $n\ (E \le E_{min})$, $n\ (E \le E_{ul})$, and E_{SX}, see text. DM_{gas} and DM_{sol} mean the dipole moment (in D) for the gas phase obtained in HF/6-31G* calculations and in solution simulations calculated from atomic point charges, respectively. [b] E_{SX} values for ions without Born correction. The correction is about −17 kcal/mol considering a radius of 9.75 Å. [c] Energy and n values were obtained with set2 instead of set1 in Table 5.

histamine result in changes of −3.74 (±0.19) and −0.88 (±0.19) kcal/mol in the solvation free energy, respectively, as averaged from the values for the forward and backward simulations. The conformational change from g3H to t3H leads to an alteration of the free energy by −1.62 (±0.18) kcal/mol. The large errors for the ΔH and the $T\Delta S$ terms preclude our concluding whether the processes are controlled by the enthalpy or the entropy change. The total relative free energies in aqueous solution are summarized in Table 7. G_{tot} values were obtained by summing up the relative values in the gas phase and those obtained by the free energy perturbation method in solution simulations. Considering four neutral species, the calculated equilibrium mixture is comprised of 83% t3H, 12% g3H, 4% t1H, and 1% g1H isomer.

Tautomerization and changes in conformations entail much larger free energy effects for the protonated species. Two simulations for the gH3+ to t3H+ conformational change in histamine led to −10.96 (±0.19) and −11.03 (±0.19) kcal/mol free energy changes using parameter set1 and set2, respectively. Thus, these different parameterizations do not affect the calculated free energy change. In contrast, the two parameter sets predict very different balance between the enthalpic and entropic contributions and emphasizes the sensitivity of the calculated ΔH and $T\Delta S$ terms to the parameter set applied. In fact, the process is entropy dominated using the original set of Jorgensen (set1) and is enthalpy dominated with set2 when the polarization within the solute is considered based on the gas-phase atomic charges (Table 5). The t3H+ to t1H+ tautomeric change leads to a relative solvation free energy of −13.14 (±0.13) kcal/mol. This result appears surprising at first sight since the formal number of the important hydration sites is 5 (3 for the NH_3^+ group and 2 around the ring) for both tautomers. In the next section structural analysis will be given for the reasons of the more favorable hydration of the t1H+ tautomer. Here we refer only to the calculated solvation characteristics that indicate the increase of the number of hydrogen bonds by 1.5 units and the more negative solute–solvent energy by 31 kcal/mol for t1H+ ($n(E \le E_{ul})$ and E_{SX} values in Table 8).

For the g3H+ to g1H+ transformation the calculated free energy change is in the range of −10.0 to −14.5 kcal/mol. The large negative value is explained by the finding that the NH_3^+ group is not in an intramolecular hydrogen bond in g1H+ and can be hydrated much better than in the g3H+ form. Fairly large hysteresis of the forward and backward simulations emerged due to both tautomeric and conformational changes ($\tau 1$ varies by 110°, Table 1) throughout the transformation. The two effects can lead to hysteresis of 0.6 and 1.8 kcal/mol separately for transformations in Table 6. The calculations seem to be less sensitive to the tautomeric changes as a local effect by developing and disappearing the tautomeric hydrogen atom than to the conformational changes where many of the solvent molecules are relocated. Taking even the more negative relative solvation free energy value the calculated G_{tot} is about 5 kcal/mol (Table 7) meaning that the g1H+ structure is far less stable than the most stable g3H+ species in aqueous solution. Calculating the equilibrium concentrations the mixture in aqueous solution comprises of 64% g3H+, 34% t1H+, and 2% t3H+.

Experimental results for the relative free energies or equilibrium fractions of the histamine tautomers/conformers cannot be

compared directly with our calculated values for the equilibrium mixture. An estimate based on potentiometric titration indicated that the 3H monocation for histamine is more stable by about 0.8 kcal/mol than the 1H form in solution.[35] Results from NMR measurements carried out on 0.4 M D_2O solution at pD = 7.9 and T = 313 led to an estimation for the equilibrium mixture of the histamine monocations in aqueous solution containing about 45% trans form.[4] Based on the ^{15}N NMR spectra for histidine with a negatively charged carboxylate side chain[36] 0.16–0.18 mole fraction for the 1H+ structure was predicted at pH ~ 8.2 and a ratio of 35:65 was found for the 1H and 3H tautomers at pH above 9. In none of these experimental results is there a clear indication for the tautomers *and* conformers in question.

Assuming that the 45% trans form estimated in ref 4 is a single trans structure and that a gauche form comprises the remaining 55% of the mixture, the trans conformer should be higher in free energy by about 0.1 kcal/mol in aqueous solution. Our theoretical estimation that the t1H+ form is higher in free energy by 0.38 (±0.23) kcal/mol than the g3H+ species is in excellent agreement with the value derived based on NMR data. Without making, however, this assumption the calculated trans fraction, 36%, is still close to the experimental value. The theoretical prediction of 66% 3H+ and 34% 1H+ tautomer is also not far from the approximately 80–20% tautomeric ratio for the protonated histamine[35] or the prediction of the 16–18% of 1H+ isomer for the structurally related histidine zwitterion.[36] This latter study predicts 1H/3H ratio of 35:65 with neutral NH_2 group (but with a negatively charged carboxylate group) as compared to our theoretical value of 5:95 for the neutral histamine isomers.

Thus, the theoretical values are in close agreement with the experimental results for histamine and in qualitative agreement with results for the structurally related but negatively charged histidine carboxylate. The calculations provide a deeper insight in the composition of the equilibrium mixture unavailable from experiment. The theoretical study also points out that the very small free energy difference of the g3H+ and t1H+ structures in aqueous solution is a balance of the large contributions, 24.5 kcal/mol in the gas phase *versus* –24.1 kcal/mol in aqueous solution. This near cancellation of the large terms explains why as high level as MP2/6-311++G** calculations in the gas phase is necessary. The t1H+ – g3H+ relative free energy in solution based on MP2/6-31G* gas-phase values is 1.56 kcal/mol, too high as compared to the experiment. Of course, our calculated free energy difference in accord with the experiment may be a fortuitous cancellation of errors made by disregarding terms higher than MP2 in the gas-phase calculations and the approximations used in the solvation calculations. Consideration of the Born correction[37] beyond the cut-off radius and effects of neglecting counterions for ionic solutes have been studied as possible sources for imprecision of the calculated values.[38] We assumed that the Born corrections, the solute–solvent interaction free energy of a point-like solute in a sphere and the continuum solvent out of the sphere, are of similar values for the isomeric species. Though the point-like solute approximation may be poor for an extended nonspherical ion *itself*, the approach must work better when comparing different isomers of the same molecule and applying a large cut-off radius (9.75 Å). The assumption of the very dilute aqueous solution and the neglect of considering the counterion seem to cause roughly additive errors and thus only little affecting relative free energies. Use of the 12-6-1 pair-additive potential seems to be satisfactory in the present case, although improvement of the binding and hydration energies for ions when using nonadditive intermolecular potentials has been demonstrated.[39]

As mentioned, we could afford only MP2/6-31G*//HF/6-31G* calculations for the dimethyl derivative in the gas phase. It was pointed out above that combining results at this level with relative solvation free energies leads to an overestimation of the total relative free energy in solution. Thus, for this molecule we considered only a conformational change of the protonated species in solution and compare this with the corresponding results for histamine. The free energy change upon the dmg3H+ to dmt3H+ conformational transformation is –9.28 ± 0.42 kcal/mol using set1. This is the average value obtained from forward and backward simulations with, however, a hysteresis of almost 4 kcal/mol. Nonetheless, the difference (1.68 kcal/mol compared to the free energy change for the g3H+ to t3H+ rotations in histamine) may be of physical origin considering the different conformations in the trans forms for histamine and dimethylhistamine and the effect of the two methyl groups. The large hysteresis in this simulation is presumed due to the larger change in the torsion angles for the dmgH3+ to dmtH3+ process. Sensitivity of the solution simulations to conformational changes were discussed in relation to the histamine structures. In the case of the dimethyl derivative the changes in the $\tau 1$ and $\tau 2$ values are 96° and 125° as compared to those of 10° and 108° for histamine, respectively.

The calculated free energy of the dmt3H+ form relative to the dmg3H+ conformer is 2.9 kcal/mol in solution (Table 7). The corresponding t3H+ – g3H+ free energy difference for the histamine monocation is 3.2 kcal/mol calculated by taking the MP2/6-31G* value for the gas-phase free energy difference in Table 3. Thus, if the same level of the ab initio calculations are used, the trans form in solution is relatively more stable by 0.3 kcal/mol for the dimethylhistamine than for histamine. If we assume a similar shift of the total relative free energy for dimethylhistamine when taking the MP2/6-311++G** gas phase value for histamine, the relative free energy of 2.1–0.3 = 1.8 kcal/mol predicts a dmg3H+/dmt3H+ ratio of 20:1 as compared to the g3H+/t3H+ ratio 33:1. Based on this simple comparison we expect the increase of the trans conformers in the equilibrium mixture of the protonated dimethylhistamine structures as compared to the mixture for histamine.

Solution Structure. Simulation results are summarized in Tables 8 and 9 and in Figures 3–5. (For interested readers supplementary material is available.) The energy pair distribution functions for methylamine and imidazole (Figure 3) are nearly additive in the –8.0 to –5.5 kcal/mol region and produce the t3H curve. This is the region characterizing the N···HwOw methylamine–water hydrogen bond[32b] and the N–H···Ow hydrogen bond for the imidazole–water system in aqueous solution.[22] The second shoulder of imidazole, which reflects the hydration of the basic nitrogen, disappears for t3H. Integrating all curves up to –2.50 kcal/mol the number of the hydrogen bonds are additive: 3 and 2 for the building block molecules and 5 for t3H. Energy pair distribution functions for g1H and g3H reflect the effect of the intramolecular hydrogen bond formation. The curve for g1H starts at –6.5 kcal/mol. Due to the N1–H···N8 intramolecular hydrogen bond the N1–H···Ow hydration of the imidazole ring and the N8···HwOw hydration of the methylamine part are prevented, thus these contributions seen in Figure 3 are missing in the g1H curve below –6.5 kcal/mol. In contrast, the curve for g3H, without a strong intramolecular hydrogen bond, is very similar to that of t3H. The number of the hydrogen bonds up to –2.5 kcal/mol are 4.7 for both gauche tautomers. The total number of the hydrogen bonds are somewhat less than for t3H, as reflected in the slightly smaller E_{SX} values (Table 8).

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Table 9. Radii for the First Shells of Hw and Ow and Coordination Numbers within[a]

	H(N)/Ow	(H)N/Ow	N/Hw	N/Ow	N_{am}/Hw	H_{am}/Ow	N_{am}/Ow
				Histamine			
g3H							
R	2.45	3.05–3.15	(2.55)	(3.35)	2.45	2.50	3.35–3.50
Coo.nb	0.9	0.8–1.0	0.7–0.9	1.2–1.3	0.6	0.8[b]	2.6–3.0
t3H							
R	2.55	3.15	2.60	3.35	2.50	2.40–2.45	3.35–3.50
Coo.nb.	1.0	1.0	1.3	1.3	1.2	0.7	2.7–3.2
g3H+							
R	2.40	3.15	nr	4.50		2.30	3.60
Coo.nb	1.0	1.1	nr	nr		1.0[b]	4.1
t3H+							
R	2.50	3.05–3.15	2.35–2.60	3.40		2.30	3.45–3.55
Coo.nb	1.1	0.8–1.0	0.5–0.8	1.5		1.0	4.5–4.6
t1H+							
R	2.50	3.10	2.60	3.35		2.20	3.45
Coo.nb	1.0	1.0	1.6	2.1		1.0	4.7
g1H+							
R	2.45	3.10	2.60	3.20		2.40	3.60
Coo.nb	1.0	1.0	1.2	1.2		1.0	4.8
				Dimethylhistamine			
dmg3H+							
R	2.50	3.15	nr	3.40		2.45	3.60
Coo.nb.	1.0	1.0	nr	1.2		1.2[b]	4.0
dmt3H+							
R	2.45	3.15	2.60	3.25–3.40		2.25–2.35	3.60
Coo.nb	1.0	1.0	0.8	0.9–1.1		1.0–1.1	4.6

[a] *R* is taken at the site of first minimum in the radial distribution function. Values in parentheses refer to poorly defined ones similarly to coordination numbers derived based on them. Abbreviation nr stands for nonrelevant. [b] Coordination number for H_{am} hydrogen bonded to N is no more than 0.3.

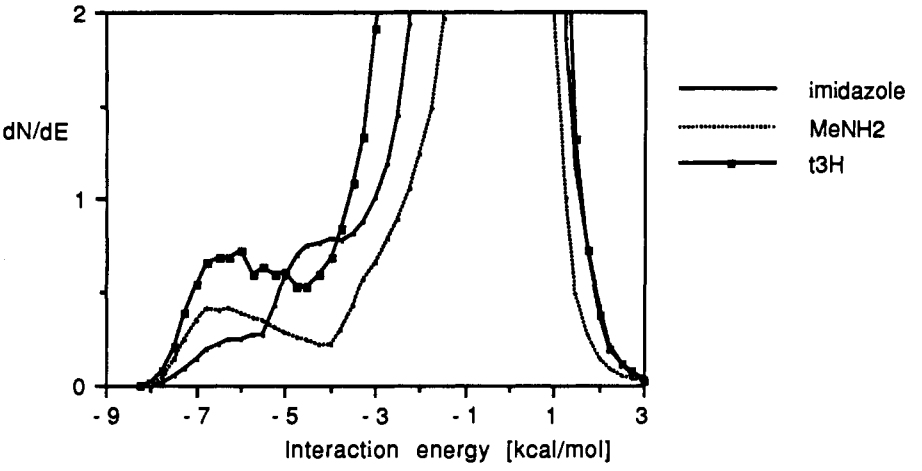

Figure 3. Energy-pair distribution functions for the t3H form of histamine (marked solid line), imidazole (solid line), and methylamine (dotted line).

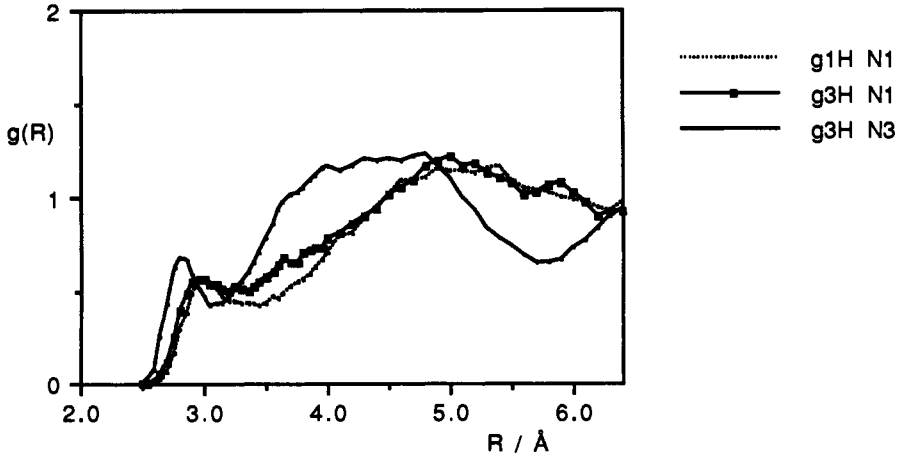

Figure 4. N···Ow radial distribution functions for g1H, N=N1 (dotted line), g3H, N=N1 (marked solid line), and g3H, N=N3 (solid line).

Energy pair distribution functions and E_{SX} values for the ionic species of the corresponding histamine and dimethylhistamine conformers are very similar. The pair distributions for the trans conformers show a definite maximum–minimum structure between −18 and −10 kcal/mol that is characteristic for the methylammonium cation. Table 8 gives 3.5 and 3.6 hydrogen

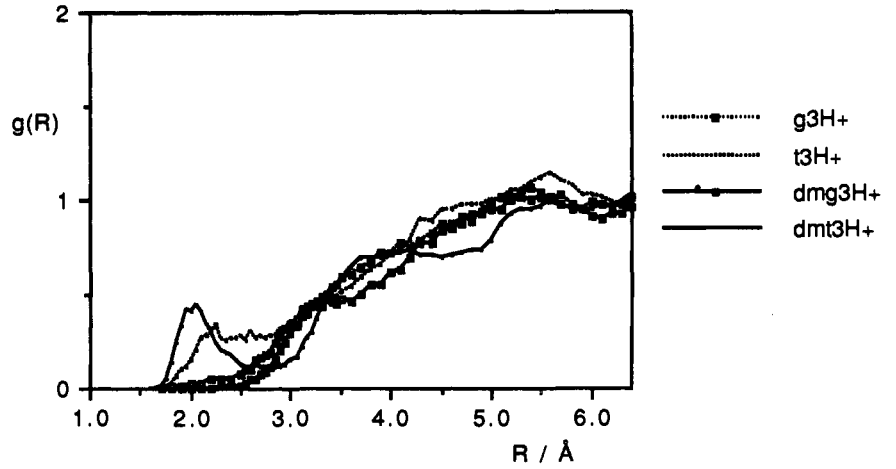

Figure 5. N1···Hw radial distribution functions for the monocations t3H+ (dotted line), g3H+ (filled squared dotted line), dmt3H+ (solid line), and dmg3H+ (empty squared solid line).

bonds for the methylammonium cation and for the t3H+ tautomers, respectively, integrating up to −10.0 kcal/mol. The gauche conformers give hydrogen bond numbers of 2.4–2.5. This reduced value reflects that one of the N8–H bonds is not open to hydration due to the strong N1···H–N8 intramolecular bond, thus one N8–H···Ow bond has been lost.

Analyses of the radial distribution functions give descriptions of the solution structure consistent with that obtained from consideration of interaction energies. The N···Ow radial distribution function (Figure 4) shows a resolved N3···Ow peak for g3H at 2.80 Å with 0.8–1.0 water molecules in the first hydration shell. The H(N)/Ow coordination number is 0.9 and the difference of the R_{max} values for H(N)/Ow and (H)N/Ow is 0.95, close to the N3–H bond length, allowing one slightly bent N3–H···Ow hydrogen bond for g3H. Because N1 is involved in intramolecular hydrogen bonds in g1H and g3H, the peaks of the N1···Ow radial distribution functions are smaller and smoother, and the minimum sites become vague. All the H(N)/Ow and (H)N/Ow rdf characteristics (location of the first nonzero rdf value, sites of maxima, and the sites of minima considered as the radii of the first shell of water Hw and Ow atoms around the specific atom) for g1H are at larger values than for imidazole indicating the water molecules are repelled out of the favorable positions to hydrate the ring system. Calculated rdf characteristics and coordination numbers for t1H and t3H indicate additivity of the hydration patterns of imidazole and methylamine thus separate and undisturbed hydrations of the two polar sites in the neutral trans conformers.

For the 3H+ species the N3···Ow radial distribution functions indicate similar hydration patterns for the histamine and dimethylhistamine structures in the N3 region. The peak heights are similar, and the R_{max} values are in a narrow range of 2.80–2.90 Å. The H(N)/Ow and (H)N/Ow coordination numbers are calculated at 0.8–1.1 and indicate one water molecule forming a localized N3–H···Ow hydrogen bond to the protonated histamines. The N1···Hw rdf's are, however, much different for the 3H+ gauche and trans forms (Figure 5). There are definite first peaks for the trans conformers (t3H+ and dmt3H+) at 2.05–2.25 Å that are completely missing for the gauche conformers. This means that the water hydrogens are repelled from the N1 neighborhood due to an H_{am} proton which forms the N1···H_{am}–N8 intramolecular bond. The shift of the R_{max} values and the decrease of the N/Hw coordination numbers from 1.4 for imidazole to 0.5–0.8 suggests that the protonated side chain has an effect on the hydration of the N1 basic nitrogen even in the trans 3H+ histamines. Some water molecules are oriented by their oxygen site facing the ring under the attractive force due to the protonated side chain and as a result the number of the N1···HwOw bonds decreases.

The rdf characteristics for g1H+ are close to those for t1H+ in the N1–H region indicating similar solution structures in the two conformations. The disruption of the intramolecular hydrogen bond upon protonation of the g1H structure is the decisive factor in determining the hydration pattern around g1H+. However, since upon this rotation the side chain points above the ring in g1H+ in contrast to the extended structure in t1H+ (Figure 2b,h) there is no room for water molecules hydrating the N3 atom of g1H+ from this side of the π region (see N3···N8 distance in Table 1). As a result, the N/Ow coordination number for g1H+ decreases to about the half of that for t1H+.

The strongest hydration site for the protonated histamines is the cationic head that is fully exposed to hydration in the trans conformation. All N_{am}···Ow peaks are at 2.80 Å, the same location as obtained for the methylammonium cation in a separate simulation. The NH_3^+ group localizes 4.5–4.8 water molecules in the first hydration shell (N_{am}/Ow coordination numbers in Table 9) for all trans conformers and for g1H+, indicating the similar hydration of the cationic head for these structures. The different heights of peaks for the gauche and trans conformers result in coordination numbers 4.0–4.1 for the g3H+ and dmg3H+ conformers. The H_{am}/Ow numbers referring to hydrogen atoms in the NH_3^+ group that are *not* in intramolecular bond even in the g3H+ and dmg3H+ structures are in the range 1.0–1.2 for all ionic isomers. This means that these hydrogen atoms are equally open to hydration or forming intermolecular hydrogen bonds to proton acceptor molecules. This conclusion will be utilized in the next section when proposing a model for the binding of the hydrated histamine to the H_2 receptor.

C_β···Ow radial distribution functions in the nonpolar region have two almost coincident first peaks for the g3H+ and t3H+ conformers at 3.70–3.75 Å, which means that the C_β atom is equally hydrated in the *gauche*- and *trans*-histamine. Hydration of this site is reduced for dmg3H+ and even more for dmt3H+. The different torsion of the ring in the dimethylhistamine *versus* histamine conformers may contribute to the C_β atom less accessible for hydration in the trans than in the gauche form.

Relationship to Histamine Receptor Activation Models. It is hoped that results from energy calculations and solution structure simulations performed in this study will contribute to an increased understanding of mechanisms of histamine receptor activation. It is certainly premature to draw far reaching conclusions concerning mechanisms of histamine H_3-receptor activation. However, it is worth considering the consequences of results from this study in relationship to existing models of H_2-receptor activation.

Weinstein et al.[13a,12d] developed a model for the activation mechanism of the H_2 receptor based on the different tautomeric preferences of the t3H and t1H histamine structures in the

protonated and neutral forms. In their mechanistic model the cationic side chain of the t3H+ form is anchored at a negative region of the receptor and "the neutralization causes a shift in the tautomeric preference to N(1)–H. N(1) could then attract a proton from a proton-donor site on the receptor while N(3) could act as a proton donor for a proton acceptor site".[12d] STO-3G calculations[12d] with molecular geometry optimization predicted the preference of the isolated t3H+ form over t1H+ and the t1H preference over t3H. In Table 3 we pointed out that all calculations using 3-21G basis set or larger predict the preference of the isolated t3H structure over the t1H tautomer both in the protonated *and* neutral form. This implies that the proton relay may not be based on the different preferences of the trans tautomers after the deprotonation of the side chain. In fact, Weinstein et al. pointed out in a later paper[13b] that explicit consideration of the receptor binding sites is necessary in the computational model to obtain a negative energy balance for the proton relay process in STO-3G calculations. Based on our calculations, a possible pathway for the Weinstein model would be that the g3H+ conformer, the predominant species in solution, gets anchored at the negative region and loses a proton at the side chain by charge neutralization. The resultant g3H structure is *less* stable than the t3H form in aqueous solution (Table 7); thus a conformational change leads to the energetically favored t3H conformer. This structure is more stable than t1H by 1.80 kcal/mol in aqueous solution disfavoring prototropic tautomerism. If dehydration at the receptor sites were to occur the free energy difference between t3H and t1H tautomers would be reduced to 0.92 kcal/mol (Table 3). Thus in the hydrated or dehydrated states the proton relay process must overcome an increase in the internal free energy.

Very recently Eriks et al.[40] proposed a four-site model for H_2-receptor activation that includes a mechanism for agonists unable to participate in a proton relay, namely isothiourea or thiazole derivatives. In their model the agonist is protonated at the nitrogen corresponding to N3 of histamine; loss of a proton results in a negatively charged receptor site III which undergoes a conformational change responsible for the activation. The positive charge is delocalized in the agonist molecule and allows a strong electrostatic interaction between the sulfur site and a hypothetical negatively charged side chain of the receptor site II. The model does not involve a proton relay for systems where it is possible, such as in the case of histamine losing proton at N1.

Our results predict an equilibrating mixture containing 64% g3H+ and 34% t1H+ isomers of histamine at physiological pH. Using the Eriks model the t1H+ structure can be anchored at a negative region of the receptor (site I) and may lose a proton forming the neutral t1H structure. This is less stable than t3H both internally and upon solvent stabilization, thus t1H, interacting with the site III of the receptor, is ready to gain a proton at N3 and to lose one at N1. This last step is not mandatory in the Eriks model but should not be ruled out for proton releasing systems. The model also considers H_2 activation by the cationic form of the agonist. The g3H+ structure interacts with the negative region of the receptor anchoring the ethylammonium cationic head and results in unfolding of the internally hydrogen bound structure. The extended t3H+ form is less stable than the t1H+ in solution, thus the latter is readily generated upon tautomeric change. This means that either the t1H+ structure (34%) or t1H+ formed from g3H+ (64%) by unfolding followed by tautomerism will be the form of histamine involved in binding to and activating the H_2 receptor. This structure can be protonated at the N3 site and may lose proton at N8 and/or N1. Our theoretical results are therefore in good agreement with all the thermodynamical requirements implicit in this model for the H_2-receptor activation.

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Conclusions

The two most stable conformers of the isolated neutral histamine molecule are gauche structures stabilized by intramolecular hydrogen bonds. The gauche 1H tautomer is more stable than the gauche 3H structure by 1.8 kcal/mol at the MP2/6-311++G**//HF/6-31G* level, considering frequency dependent corrections for the free energy at 298 K. Trans conformers at this level are higher in free energy by 2.2–3.2 kcal/mol. The free energies of the monocation protonated at the side chain are higher in the t3H+, g1H+, and t1H+ forms by 13–25 kcal/mol than the protonated gauche 3H conformer. (αR,βS)-α,β-Dimethylation of histamine has an effect mainly on the equilibrium geometries leaving the energy sequence and separations of the conformers close to those obtained for histamine.

In contrast to the gas phase, in aqueous solution at pH greater than 9–10 the trans 3H histamine is the most abundant neutral conformer of those considered here, comprising 83% of the equilibrium mixture. The preference for this conformer over the gauche forms may be attributed to the larger number of polar sites available for hydration in aqueous solution. Protonated structures prevailing at physiological pH exhibit an equilibrium of the internally bound g3H+ structure (64%) and the extended t1H+ (34%) and t3H+ (2%) forms. The calculated fraction of the trans conformers is 36%, close to the experimental estimate of 45%. The fraction of the 3H+ tautomers was calculated at 66% as compared to the experimental value of about 80%. The free energy difference favoring the g3H+ structure over the t3H+ form of the monocationic (αR,βS)-α,β-dimethylhistamine in aqueous solution is somewhat less than that for the histamine itself. The theoretical calculations point out that the resulting small free energy difference among the monocationic isomers in the aqueous solution is due to a delicate balance of the strong internal stabilization *via* intramolecular hydrogen bond and the considerable solvent effect preferring the larger number of hydratable sites with stronger hydrogen bonds.

Energy calculations and solution structure simulations allow proposals of pathways for the binding process of the histamine isomers from aqueous solution to the H_2 receptor. Following the Weinstein model the g3H+ form is anchored at the negative receptor region. After deprotonation a conformational change leads to the t3H conformer that is the most stable neutral form in aqueous solution. Depending on the hydration state, the t3H internal free energy is lower by 0.9–1.8 kcal/mol than that for the t1H tautomer, the product of the proton relay process in the Weinstein model. Following the recent proposal of Eriks et al., the t1H+ isomer can be readily anchored and protonated at N3 of the imidazole ring in the bound conformation. If the system produces a proton relay in its neutral form the resulting t3H is favored by the present thermodynamic calculations.

Supplementary Material Available: Solution structure analysis, a detailed table of rdf's characteristics, and five further figures for pair-energy and radial distribution functions (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.