Concentration and basicity of histamine rotamers

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A method was developed and applied to determine the populations and site- and conformer-specific basicities of histamine rotamers in three distinct states of protonation. The method that has been developed also allows the determination of molecule- and position-specific standard *gauche* and *trans* ^{1}H NMR coupling constants, and the subsequent rotamer analysis from NMR spin systems with a single observed coupling constant, introducing simplifying allowances of symmetry origin. Synthesis and NMR analysis of a reference histamine derivative have also been carried out. The *trans* rotamers of histamine were found to exist in 41, 38 and 50% in the neutral, monocationic and dicationic forms, respectively, providing quantitative, experimental evidence for the preferential thermodynamic properties of the *trans* species over the two enantiomeric, statistically favoured g conformers in any ionisation form. Quantification of the rotamer- and site-specific basicities resulted in increased amino and decreased imidazole basicities in the *gauche* rotamer, indicating the formation of intramolecular hydrogen bonds in the monocationic form.

Introduction

Histamine, the small, versatile biogenic amine is one of the most thoroughly studied chemical entities, due to its enormous biological significance. Its biochemical functions are mediated mainly *via* histamine receptors,[1,2] of which four different types have been identified so far. The exogenous, specific H_1, H_2, *etc.* agonists and antagonists are ligands of one particular receptor only, unlike histamine, the endogenous activator of every histamine receptor. This indicates that histamine binds to the H_1, H_2, *etc.* receptors in different conformations and/or forms of ionisation. Characterisation of the various conformational, ionisable forms is therefore an important step in elucidating the molecular background to the biological versatility.

Despite its mere 8 skeleton atoms, histamine occurs in a variety of structural forms.[3] The amino and imidazole moieties are differently and interactively basic, *i.e.* protonation at one site significantly reduces the basicity of the other site. The molecule therefore takes up several forms of protonation. The imidazole ring undergoes tautomeric changes, the extent of which depends on the molecular state of ionisation. Also, the imidazole C(2) proton is one of the few carbon bonded acidic protons.[4] Histamine is considered an achiral, prochiral compound. Its prochirality becomes evident if any of the methylene geminal hydrogens is substituted. Such substitution can be a histamine⟶histidine conversion, or simply, a deuterium–hydrogen replacement. Equivalence of the geminal hydrogens results in identical concentrations of stereochemically distinct species, which allows simplified treatment of some conformer-specific parameters, as shown in the Results and discussion section. The sigma bonds in the aliphatic side-chain permit rotation, giving rise to different conformations of the molecule. Due to the above properties, histamine exists in solution as a mixture of several ionic, tautomeric and conformational forms of short individual lifetime and rapid interconversion. These species, however, have their own physicochemical properties, and they act individually and specifically in stereochemically controlled biochemical processes.

The protonation equilibria of histamine have been described at the macroscopic[5,6] and microscopic levels.[7] In aqueous solution at 37 °C and physiological pH (7.4) the major species bears one extra proton at the amino site, while the imidazole ring occurs at 80% in the N(τ)–H tautomer.[8,9] At lower pH (still present in living systems) the dicationic species predominates. Solid phase X-ray crystallographic studies proved the presence of the *trans* conformation in all three protonation states.[10–12] Nevertheless, the *trans* and *gauche* conformers coexist in solution, and their ratios in the monocationic and neutral forms have been found to be nearly equal,[13,14] as determined by ^{1}H NMR spectroscopy in D_2O. Theoretical studies have also been carried out to predict preferential conformations in the gas phase and solution.[13,15–19] The reported values reflect method-dependent energy differences, in which *ab initio* and Monte Carlo studies[18] showed preferential *gauche* conformers, whereas AMBER force field methods[19] resulted in a higher concentration of the *trans* rotamer.

The H_1, H_2, H_3 and H_4 receptors and their selectivity towards exogenous ligands assume four different, active conformations of histamine. Their binding stereochemistry could be characterised by docking studies from knowledge of the three-dimensional (3D) structures of the receptor pockets. Although significant progress has been achieved in elucidating the amino acid sequence and other molecular properties of histamine receptors,[20–23] the 3D structures have not yet been reported. Conclusions on the binding forms of histamine can therefore be drawn from structure-dependent activity investigations of synthetic histamine derivatives, including the exogenous agonists and antagonists. Such investigations suggest the fitting of the extended *trans–trans* conformation of the N(τ)–H histamine monocation to H_1 and H_2 receptors.[24–28] The capability of undergoing 1–3 prototropic tautomerism was assumed to be necessary for H_2 activity.[26] Much less is known about the stereochemical requirements on the H_3 receptor, but existence of the *gauche–trans* conformer has been hypothesised.[28,29] No data has appeared on the stereochemical aspects of the histamine–H_4 complex. The stereochemistry of histamine species is also important in metal complexation,[30] and heparin-bound storage in mast cells.[31]

Since the biological function of any of the species cannot be ruled out, and the reactive species is not necessarily the major one,[4] we determined the concentration of every significant, coexisting histamine species in aqueous solution. Rotamer populations were determined for the three protonation states by an improved, molecule-specific version of ^{1}H NMR coupling

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constant analysis. The acid–base properties of every conformer have also been quantified.

Experimental

Histamine dihydrochloride, deuterium chloride, and sodium deuteroxide were obtained from Sigma Chemical Co. Deuterium oxide was obtained from Merck. The 0.1 M histamine solutions were prepared in 10% D_2O and 90% H_2O mixtures, 2 M DCl–HCl and 2 M NaOD–NaOH 10 : 90 solutions were used to set the pH.

pH Measurements were carried out with a Radiometer PHM93 pH meter, equipped with an Orion 9103BN semi micro combined glass electrode. The electrode was calibrated using aqueous (pH = 4.003 and 9.198, 296 K) buffer solutions. ^{1}H NMR studies were carried out using Bruker AM 360 and Bruker AM 200 spectrometers. Protonation constants of histamine were determined by ^{1}H NMR–pH titrations in solutions of 16 different pH values, ranging from 3.5 to 13, and at 296 K. Chemical shifts were measured relative to internal *tert*-butyl alcohol (1.236 ppm). For the determination of the vicinal proton–proton coupling constants of histamine, NMR spectra were recorded at pH = 3.5, 8.0, and 13, where the three distinct protonation states of the molecule exist almost exclusively. Coupling constants were obtained from spectra of 0.02 Hz digital resolution.

For the determination of imidazole substituent constants, 4-isopropylimidazole was synthesised. As a first step 1-bromo-3-methylbutan-2-one was prepared from 3-methylbutan-2-one (Fluka) by the method of McMorris *et al*.[32] This substance was then condensed with formamidine acetate to form 4-isopropylimidazole in a similar manner to the literature method.[33,34] After isolation and purification of the product by TLC, its structure was proved by NMR. ^{1}H NMR coupling constants of 4-isopropylimidazole were recorded in D_2O at pD < 2 and pD > 10.

Results and discussion

Rotamer populations

Fig. 1 shows the numbering of the histamine molecule and its

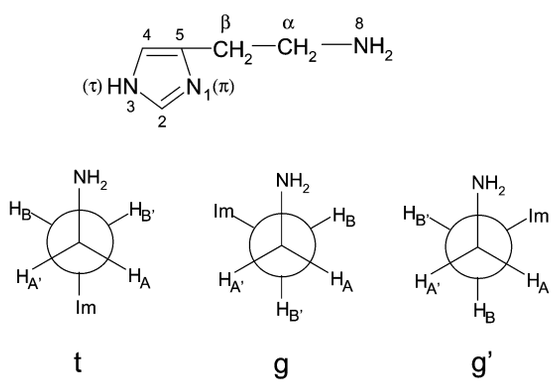

Fig. 1 Histamine: numbering and designation of its atoms and the staggered forms of its rotamers.

staggered conformers, where the rotational axis is the α–β carbon–carbon bond. Rotamer analyses have been carried out on the basis of the Karplus relationship and vicinal coupling constants.[35–37] The vast majority of such analyses used the observed ^{1}H NMR coupling data on spin systems with 3 coupling protons, and two observed non-identical vicinal coupling values. Such data sets allowed the calculation of the rotamer populations using standard, *trans* and *gauche* coupling constants irrespective of the type of molecule.[35–37] As an improvement on rotamer analysis we used molecule- and position-specific *trans* and *gauche* constants, calculated by means of moiety electronegativities introduced by Altona *et al*.[38]

The ^{1}H NMR spectrum of histamine contains the information of a single observed vicinal coupling value only. Rotamer populations could thus be obtained by a modified version of the analysis, in which molecular symmetry has been taken into account in the process of evaluation.

The four aliphatic protons of histamine form an AA′BB′ spin system where A and A′, B and B′ protons are chemically equivalent. Rotation about the α–β carbon–carbon bond is rapid on the NMR time-scale. The AA′BB′ spin system appears as two symmetrical triplets, indicating the identical NMR behaviour of conformers g and g′, the mirror image species. Thus, the conformational equilibrium of histamine can be characterised by the ratio of two rotamers, where the occurrence probability of rotamer g is two times higher than that of rotamer t.

The observed vicinal proton–proton coupling constant of histamine is a weighted sum of the various *gauche* and *trans* coupling constants of individual rotamers, where weighting factors are the appropriate mole fractions:

$$^3J_{HH} = \alpha_t \frac{2J_T + 2J_{Gt}}{4} + \alpha_g \frac{J_T + J_{Gg_{A'B'}} + J_{Gg_{B'A}} + J_{Gg_{AB}}}{4} \quad (1)$$

$$\alpha_t + \alpha_g = 1 \quad (2)$$

In eqns. (1)–(2), $^3J_{HH}$ is the observed coupling value of histamine, α_t and α_g are rotamer populations, J_T is the standard coupling constant corresponding to a 180° dihedral angle, J_{Gt}, $J_{Gg_{A'B'}}$, $J_{Gg_{B'A}}$ and $J_{Gg_{AB}}$ are standard coupling constants corresponding to 60° and 300° dihedral angles in rotamers t and g, respectively, as shown in Fig. 1.

By combining eqns. (1)–(2) we obtain:

$$\alpha_t = \frac{4^3J_{HH} - J_T - J_{Gg_{A'B'}} - J_{Gg_{B'A}} - J_{Gg_{AB}}}{J_T + 2J_{Gt} - J_{Gg_{A'B'}} - J_{Gg_{B'A}} - J_{Gg_{AB}}} \quad (3)$$

Eqn. (3) shows that the determination of rotamer populations uses the standard coupling constants. Such parameters are not '*a priori*' known. In order to determine their molecule- and species-specific values we calculated the J_T, J_{Gt}, $J_{Gg_{A'B'}}$, $J_{Gg_{B'A}}$ and $J_{Gg_{AB}}$ quantities from the reparametrised Karplus equation of Altona *et al*.[38] (Table 1). Using this equation and empirical group electronegativities (substituent constants) we could take not only the type of group, but also the effect of its protonation into consideration. Note that this method provides four *gauche* and one *trans* standard constant for every protonation state. Calculation of the different J_{Tt} and J_{Tg} parameters, which exist theoretically, resulted in identical values. Substituent constants have been reported by Altona *et al*.[38] for the amino and ammonium groups. No analogous literature data was available for the imidazole and imidazolium sites. We therefore measured the coupling constants of 4-isopropylimidazole. This compound was suggested for such studies by Altona, since the coupling constants of isopropyl derivatives are independent of the torsion angles. The values obtained for 4-isopropylimidazole are $J = 6.90$ Hz for the basic form, and $J = 6.93$ Hz for the protonated form. Calculations for the imidazole ring with a side-chain in the C(5) position resulted in substituent constants 0.50 and 0.47 for the neutral and cationic forms of imidazole, respectively. The slight difference can be interpreted by the enhanced electron-withdrawing effect of the imidazolium moiety, and also by the fact that the positive charge spreads over the ring.

The standard coupling constants were obtained by the Altona equation (rms error: 0.36 Hz).[38] This allowed the calcu-

Table 1 Observed and standard vicinal ^1H–^1H NMR couplings and rotamer populations of histamine

Protonation state	Observed couplings $^3J_{HH}$/Hz	Standard *trans* and *gauche* coupling constants[a]/Hz					Rotamer populations	
		J_T	J_{Gt}	$J_{GgA'B'}$	$J_{GgB'A}$	J_{GgAB}	a_t	a_g
NH_2–Im	6.84	13.19	4.19	2.76	2.45	3.88	0.41	0.59
NH_3^+–Im	6.97	13.57	4.05	3.22	2.90	3.73	0.38	0.62
NH_3^+–Im^+	7.35	13.62	4.04	3.21	2.93	3.76	0.50	0.50

[a] Calculated by Altona's equation (rms = 0.36 Hz).[38]

lation of the rotamer mole fractions according to eqns. (2)–(3). Error-proliferation calculations resulted in 0.07 and 0.10 uncertainties in the a_t and a_g values, respectively. Table 1 shows that concentration of the g conformer exceeds that of the t conformer in the neutral and monocationic forms. In the dicationic form the t and g populations are equal. Considering the twofold occurrence probability of the *gauche* conformation, it can be stated that rotamer t is the energetically more stable rotamer. This phenomenon can be interpreted in steric terms, since rotamer t contains bulky groups in the remote *trans* position. Furthermore, water accessibility to the amino or ammonium moieties is also favourable in the *trans* position.[39] Rotamer t is most populated in the dicationic form, a consequence of the electrostatic repulsion between the two positively charged groups. The relatively higher mole fraction of rotamer g in the neutral and monocationic histamine can be explained by the lack of cation–cation repulsion, but also by the stabilising effect of intramolecular hydrogen bonds between the amino and imidazole groups. There is no significant difference between rotamer populations in these protonation states, since the major factors governing the formation of conformers are similar in these cases.

Rotamer-specific basicities

Fig. 2 shows the bulk and rotamer-specific protonation equilibria of histamine.

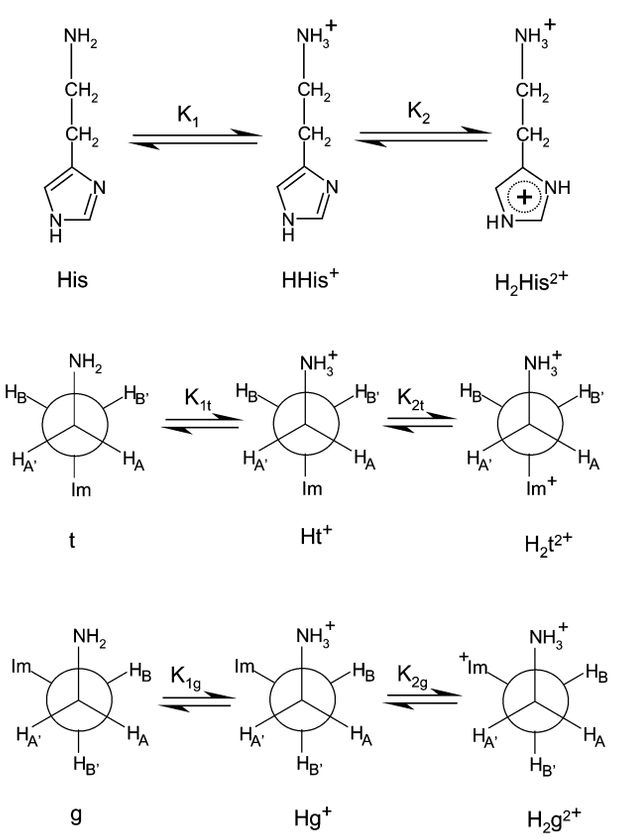

Fig. 2 Bulk and rotamer-specific protonation equilibria of histamine.

Table 2 Parameters of the non-linear parameter fitting

Observed proton	δ_{His}/ppm	δ_{HHis^+}/ppm	$\delta_{H_2His^{2+}}$/ppm	R
Imidazole-2-H	7.667	7.723	8.669	0.9997
Imidazole-4-H	6.911	7.043	7.404	0.9998

libria. Macroscopic protonation constants can be expressed in terms of the concentrations of macrospecies:

$$K_1 = \frac{[HHis^+]}{[His][H^+]} \qquad K_2 = \frac{[H_2His^{2+}]}{[HHis^+][H^+]} \tag{4}$$

The observed ^1H NMR chemical shifts depend on pH (Fig. 3)

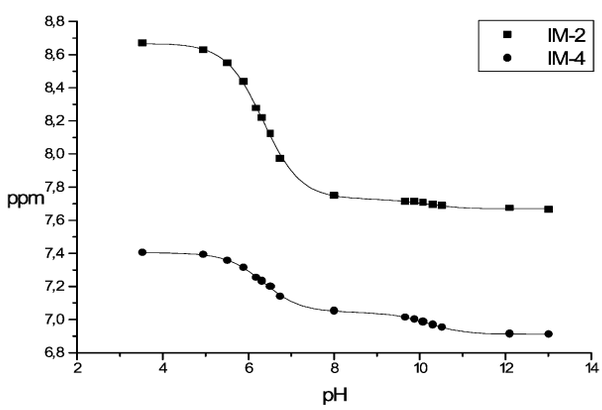

Fig. 3 Chemical shift–pH functions of imidazole C-2 and C-4 protons of histamine.

and can be formulated as follows:

$$\delta_{obs} = \delta_{His}a_{His} + \delta_{HHis^+}a_{HHis^+} + \delta_{H_2His^{2+}}a_{H_2His^{2+}} \tag{5}$$

where a values are exemplified by $a_{H_2His^{2+}}$ in eqn. (6)

$$\alpha_{H_2His^{2+}} = \frac{[H_2His^{2+}]}{[His]+[HHis^+]+[H_2His^{2+}]} = \frac{K_1K_2[H^+]^2}{1+K_1[H^+]+K_1K_2[H^+]^2} \tag{6}$$

From NMR–pH titration curves of the imidazole protons the macroscopic protonation constants were determined by non-linear parameter fitting and are in agreement with literature data.[5–7] The macrospecies-specific chemical shifts and correlation coefficients of the non-linear parameter fitting are listed in Table 2.

Definition and evaluation of the rotamer-specific basicities is exemplified below by rotamer t

$$t + H^+ \rightleftharpoons Ht^+ \tag{7}$$

$$K_{1t} = \frac{[Ht^+]}{[t][H^+]} \tag{8}$$

Table 3 Macroscopic and rotamer-specific protonation constants of histamine

$\log K_1 = 10.16$	$\log K_2 = 6.35$
$\log K_{1t} = 10.12$	$\log K_{2t} = 6.47$
$\log K_{1g} = 10.18$	$\log K_{2g} = 6.26$

where K_{1t} quantifies the t-rotamer-specific amino basicity of histamine.

Rotamer concentrations can be expressed with macroscopic concentrations and rotamer mole fractions:

$$[t] = [His]\alpha_t \quad (9)$$

$$[Ht^+] = [HHis^+]\alpha_{Ht^+} \quad (10)$$

Introducing eqns. (9)–(10) into eqn. (8) yields:

$$K_{1t} = \frac{\alpha_{Ht^+}[HHis^+]}{\alpha_t[His][H^+]} = \frac{\alpha_{Ht^+}}{\alpha_t} K_1 \quad (11)$$

Logarithmic values of the macroscopic and rotamer-specific protonation constants are collected in Table 3. Although macroscopic constants, in principle, cannot be assigned to specific sites, constants with subscripts 1 and 2 practically purely characterise the basicity of the amino and imidazole groups, respectively.

The rotamer-specific amino protonation constants differ insignificantly from each other and the respective bulk constant. The imidazole protonation exerts a more significant effect on the rotamer populations. The low $\log K_{2g}$ value shows that uptake of a second proton in the *gauche* rotamer brings about breakdown of the monocationic hydrogen bond, and this process needs a relatively high hydrogen ion concentration. The evaluated $\log K_{2t}$ value mainly reflects the basicity of the imidazole moiety, which is undisturbed by intramolecular hydrogen bonds, but is certainly influenced by the skeleton-mediated electron-withdrawing effect of the adjacent ammonium site. The above statements support our observations on the amino basicity of rotamers. The higher $\log K_{1g}$ value indicates that the imidazole group promotes the amino protonation by intramolecular, inter-moiety hydrogen bond formation in *gauche* amino-imidazole positions only.

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