BIOORGANIC CHEMISTRY 12, 34–44 (1983)

Ionization Constants and Thermodynamic Parameters of Histidine and Derivatives

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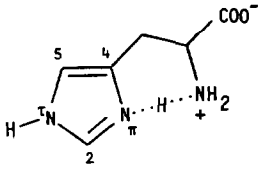
Received April 28, 1983

The pK_a values for the proton dissociation of carboxyl, imidazolium, and ammonium groups for histidine and ten of its derivatives were determined electrometrically at seven temperatures in the range 10–40°C. The ΔH and ΔS values were estimated from the temperature dependence of the dissociation constants of histidine and its derivatives. These results and the pK_a values compared in terms of inductive effect suggest an ion–dipole interaction between the protonated amino group and the unprotonated imidazole ring. The charge and the solvation effects of the neighboring groups are the main factors that determine the imidazole group pK_a in histidine and its studied derivatives. The N^{τ}-H tautomer is favored over the N^{π}-H by 1.6 kcal/mol, indicating that the inductive substituent effect at position 4 of the imidazole ring is the major component in determining this tautomeric preference.

INTRODUCTION

Histidine plays important part as structural and functional component in determining the mode of action of many proteins (*1, 2*) and natural peptides (*3–5*). The titration of histidine, histidine derivatives, and histidine-containing peptides has been done in order to gain information on the surroundings of the imidazole group (*6, 7*) and on the conformation of peptides (*8, 9*). The pK_a of imidazole and other titratable groups in histidine and related compounds have been determined by electrometric titration (*10–13*) as well as by resonance methods such as ^{1}H nmr (*6, 7, 14*), ^{13}C nmr (*15*), and ^{15}N nmr (*16–18*). Some of these studies led to the proposal of a hydrogen bond in histidine between the protonated α-amino group and the π-nitrogen (Scheme 1) in aqueous solution (*12, 14, 16*) as well as in the solid state (*17*). The same hydrogen bond was proposed for histamine to explain,



SCHEME 1

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in part, its pharmacological activity (*19, 20*) and more recently to justify the N^{τ}-H tautomer preference of the imidazole ring (*18*). On the other hand, electrometric titration and its thermodynamic parameters (*21*) and also theoretical studies (*22*) did not support this hydrogen bond for histamine. Previous work has shown that the positively charged α-amino group decreases the reactivity of the imidazole ring of histidine towards *p*-nitrophenylacetate, and this effect was thought to be due to an ion–dipole interaction between the α-ammonium group and the unprotonated imidazole ring (*23*).

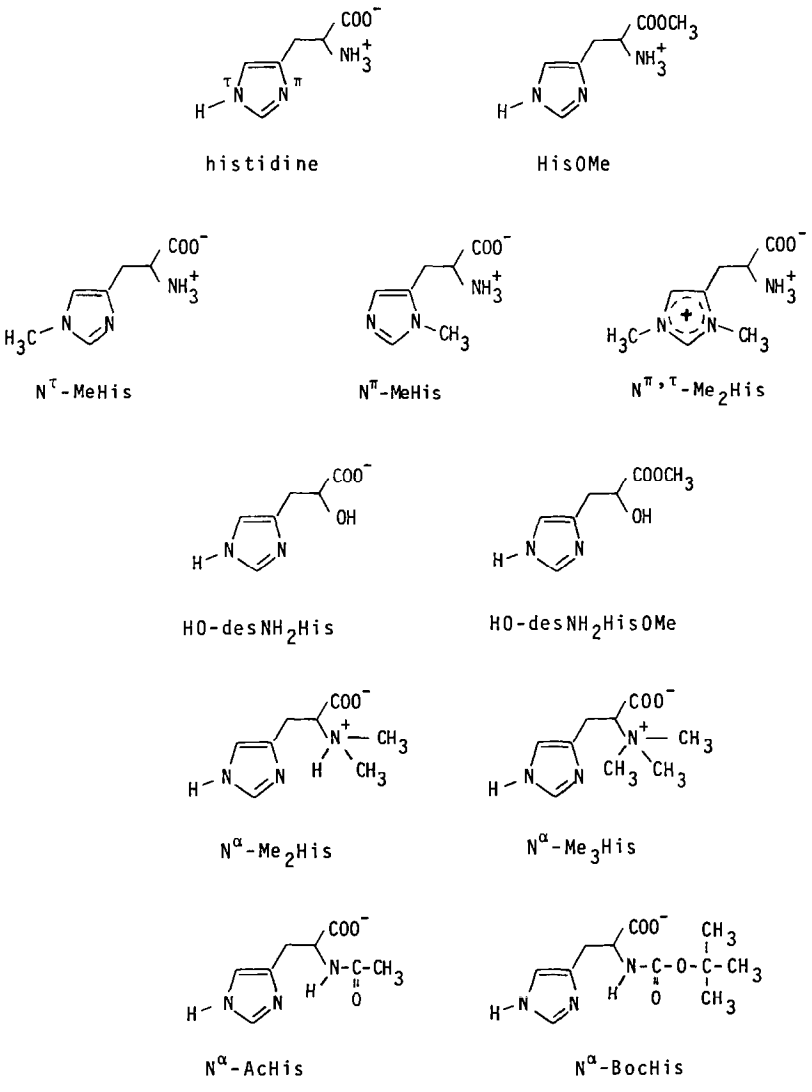
The acylation of the α-amino group and the esterification of the carboxyl group affect histidine's imidazole pK_a value to different extents, indicating that the positively charged α-amino group has a greater effect than the negatively charged α-carboxyl group on that group's pK_a, and the reason for this is not entirely clear (*6, 7, 24*). In order to obtain further information about the interaction of the imidazole group of histidine with its neighboring groups, and to evaluate the energetics of these processes, we determined the pK_a values and the corresponding thermodynamic parameters (ΔH and ΔS) by doing titrations, at seven different temperatures, of the following compounds:[1] histidine, histidine methyl ester (HisOMe), N^{τ}-methyl histidine (N^{τ}-MeHis), N^{π}-methyl histidine (N^{π}-MeHis), $N^{\pi,\tau}$-dimethyl histidine ($N^{\pi,\tau}$-Me_2His), α-hydroxydesamino histidine (HO-desNH_2His), α-hydroxydesamino histidine methyl ester (HO-desNH_2HisOMe), N^{α}-dimethyl histidine (N^{α}-Me_2His), N^{α}-trimethyl histidine (N^{α}-Me_3His), N^{α}-acetyl histidine (N^{α}-AcHis), and N^{α}-t-butyloxycarbonyl histidine (N^{α}-BocHis) (Scheme 2).

EXPERIMENTAL

The following compounds were obtained from commercial sources: L-histidine from Calbiochem; N^{π}-MeHis, N^{τ}-MeHis, and N^{α}-AcHis from Sigma Chemical Company. They were found to be homogeneous by TLC on silica gel with three different solvent systems and by paper electrophoresis at pH 5, and they were used without further treatment. The following compounds were synthesized as previously described (*23*): HisOMe, N^{α}-BocHis, N^{α}-Me_2His, N^{α}-Me_3His, $N^{\pi,\tau}$-Me_2His, HO-desNH_2His, and HO-desNH_2HisOMe. The electrometric titrations and the treatment of titration data were done as described elsewhere (*9, 25*). The titrations were done at seven temperatures in the range 10–40°C, in 0.15 *M* KCl, with more than 40 points for compounds with one titratable group and with more than 80 points for those with two or more titratable groups.

The errors associated with the pK_a values were estimated by statistical treatment of at least four titrations at each temperature and the maximum values for 95% fiducial limits were ± 0.1 for pK_a values below 2.5 and ± 0.03 for pK_a values in the range 5–9.5. An example of the fit of the experimental points to the theoretical

[1] To avoid the ambiguity in the nomenclature of the *N*-methyl histidines, due to conflicting numbering systems for the imidazole ring, the imidazole nitrogens have been named *pros* (π) and *tele* (τ), according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, cf. *Biochemistry* **11,** 1726 (1972). In the X-ray crystallographic numbering system N^{π} corresponds to ND1 and N^{τ} to NE2.



SCHEME 2

curve representing the sum of Henderson–Hasselbalch terms is shown in Fig. 1. The ΔH values were estimated from linear equations adjusted by the method of least squares to the dependence of pK_a on $1/T$ with correlation coefficient no less than 0.980 for imidazole and amino groups. For carboxyl group pK_a values, which showed low temperature dependence, the correlation coefficients ranged from -0.8 to 0.6.

The standard errors of the ΔH values were obtained from the standard errors of the slopes of the pK_a vs $1/T$ curves, and the standard errors associated with ΔS values were calculated taking into account the propagation of the errors in ΔG and ΔH.

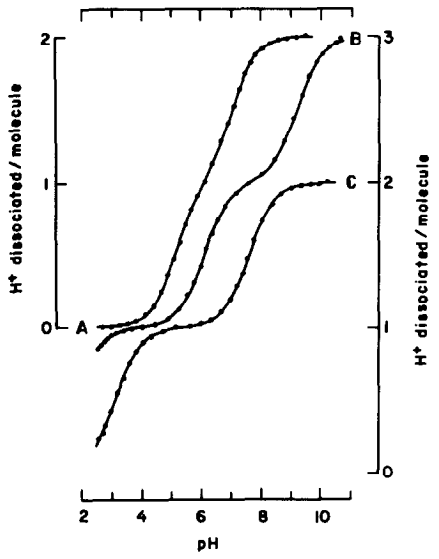


FIG. 1. Titration curve in 0.15 *M* KCl for HisOMe (A) at 40°C, histidine (B) at 25°C, and OH-desNH$_2$His (C) at 15°C. The points represent experimental data from one titration and the curve is the theoretical one calculated with the following pK_a values: (**A**) 5.18 and 7.03; (**B**) 1.80, 6.14, and 9.20; (**C**) 3.12 and 7.62.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 show the pK_a values obtained at 25°C, as well as the ΔH and ΔS values in the range 10–40°C.

TABLE 1

TITRATION DATA FOR THE CARBOXYL GROUP OF HISTIDINE AND DERIVATIVES AT 25°C IN 0.15 M KCl

Compound	pK_a	$\Delta H^{a,b}$ (kcal/mol)	ΔS (25°C)^b (e.u.)
His	1.80	−0.4 ± 0.5	−9 ± 2
HO-desNH$_2$His	3.12	−0.6 ± 0.4	−16 ± 1
N$^{\alpha}$-AcHis	2.97	0.2 ± 0.2	−13 ± 1
N$^{\alpha}$-BocHis	3.10	−0.1 ± 0.3	−15 ± 1
N$^{\alpha}$-Me$_2$His	1.15	0.3 ± 0.5	−4 ± 2
N$^{\alpha}$-Me$_3$His	1.10	0.6 ± 1.0	−3 ± 3
N$^{\pi}$-MeHis	1.78	0.2 ± 0.5	−7 ± 2
N$^{\tau}$-MeHis	1.87	−0.1 ± 0.4	−9 ± 1
N$^{\pi,\tau}$-Me$_2$His	1.98	−0.2 ± 0.5	−8 ± 2

[a] Obtained from the best fit of the data to van't Hoff's equation by the method of least squares.

[b] Errors indicated are standard deviations.

TABLE 2

TITRATION DATA FOR THE IMIDAZOLE GROUP OF HISTIDINE AND DERIVATIVES AT 25°C in 0.15 M KCl

Compound	pK_a	$\Delta H^{a,b}$ (kcal/mol)	ΔS (25°C)^b (e.u.)
His	6.14	6.8 ± 0.2	−5.4 ± 0.7
HO-desNH$_2$His	7.43	6.7 ± 0.2	−11.5 ± 0.7
N$^\alpha$-AcHis	7.11	7.8 ± 0.1	−6.3 ± 0.4
N$^\alpha$-BocHis	7.18	7.6 ± 0.3	−7.3 ± 0.9
HisOMe	5.43	7.2 ± 0.1	−0.6 ± 0.3
HO-desNH$_2$HisOMe	6.85	7.3 ± 0.3	−6.8 ± 1.1
N$^\alpha$-Me$_2$His	6.15	7.0 ± 0.1	−4.8 ± 0.3
N$^\alpha$-Me$_3$His	6.12	6.8 ± 0.1	−5.2 ± 0.5
N$^\pi$-MeHis	6.61	7.4 ± 0.2	−5.3 ± 0.5
N$^\tau$-MeHis	5.99	5.8 ± 0.1	−7.9 ± 0.3

^{a,b} The same meanings reported in Table 1.

The previously reported pK_a values for the carboxyl, imidazole, and amino groups of histidine (*10, 13*), HisOMe (*6, 13*), N$^\alpha$-AcHis (*6*), N$^\pi$-MeHis (*11*), N$^\tau$-MeHis(10), and the values presented here are in good agreement, as well as the ΔH and ΔS values for histidine and HisOMe (*13*). The titration data for the other histidine derivatives studied here have not been reported before.

Carboxyl groups (Table 1). The acylation of the α-amino group (N$^\alpha$-AcHis and N$^\alpha$-BocHis) or its replacement by a hydroxyl group increases the carboxyl group pK_a by 1.3 units, but the alkylation of the α-amino group (N$^\alpha$-Me$_2$His and N$^\alpha$-Me$_3$His) reduces it by 0.7 units. These chemical modifications cause ΔG changes mainly by entropy contribution, since ΔH values for all the compounds listed on Table 1 are very similar. This suggests that substitutions near the carboxyl function affect its ionization not only by inductive effect but also by alteration of the solvent structure around the carboxyl group. These results are in agreement with those obtained from dissociation studies of α,ω-amino acids(26) and discussed for

TABLE 3

TITRATION DATA FOR THE AMINO GROUP OF HISTIDINE AND DERIVATIVES AT 25°C IN 0.15 M KCl

Compound	pK_a	$\Delta H^{a,b}$ (kcal/mol)	ΔS (25°C)^b (e.u.)
His	9.20	10.3 ± 0.1	−7.7 ± 0.2
HisOMe	7.36	10.0 ± 0.2	−0.3 ± 0.6
N$^\pi$-MeHis	8.73	9.8 ± 0.1	−7.2 ± 0.4
N$^\tau$-MeHis	9.27	11.1 ± 0.1	−5.2 ± 0.2
N$^{\pi,\tau}$-Me$_2$His	7.85	9.8 ± 0.2	−3.2 ± 0.8
N$^\alpha$-Me$_2$His	8.98	6.1 ± 0.1	−20.8 ± 0.4

^{a,b} The same meanings reported in Table 1.

its *N*-substituted derivatives(27), where for $n \leq 3$ the Kirkwood–Westheimer treatment fails to describe the effect of the positive ammonium group on the pK_a of the carboxyl group.

The alkylation of π or τ nitrogens (N^{π}-MeHis and N^{τ}-MeHis) or both ($N^{\pi,\tau}$-Me_2His) does not interfere significantly with the dissociation parameters of histidine's carboxyl group.

Imidazole groups (Table 2). The acylation of the α-amino group (N^{α}-AcHis and N^{α}-BocHis) increases the pK_a of histidine's imidazole group by 1 unit while the substitution of the α-amino by a hydroxyl group (HO-desNH_2His) raises the imidazole pK_a by 1.3 units. The acid-strengthening effect of the ammonium group over the imidazole is indicated by the larger ΔH values for the acylated derivatives as compared to histidine. On the other hand the higher pK_a of HO-desNH_2His is wholly due to the more negative ΔS, indicating a significant solvent effect caused by the substitution of OH for NH_3^+.

The esterification of the carboxyl group decreases the imidazole pK_a by 0.7 units in histidine (HisOMe) and by 0.58 units in HO-desNH_2His (HO-desNH_2 HisOMe). These results show that the presence of an adjacent negatively charged carboxyl group has a smaller effect in raising the imidazole pK_a than the presence of an adjacent positively charged amino group in lowering it. The same effect is observed comparing the pK_a of histamine (6.4) (*21*) and imidazole-4-propionic acid (7.7) (*17*) with that of 4-methyl-imidazole (7.52) (*28*). The reason for this difference is not clear, although it has been attributed to delocalization of electronic charge in the carboxyl group due to its resonance (*6*). The data of Table 2 show that the lower pK_a values of the methyl esters relative to the parent compounds are accounted for by higher (less negative) ΔS values, without contribution from ΔH, whose values are even higher in the esters than in the parent compounds.

As a result of ^{1}H nmr (*14*) and ^{15}N nmr (*16*) studies of histidine and some of its derivatives, an ion–ion interaction between the carboxylate and imidazolium groups has been proposed for histidine in aqueous solution in the pH range 2–6. The absence of the carboxyl–imidazolium interaction in HisOMe therefore has two opposite effects on the imidazole pK_a: one is electrostatic, which tends to lower it, and the other is by increasing solvation of the imidazole, as suggested by the imidazole ΔS value in HisOMe, which tends to raise the pK_a. Below pH 6.2 the acylation of the α-amino group or its substitution by a hydroxyl group would not disrupt any ion–ion interaction, so that just the effect of the positively charged group is observed.

A semiquantitative idea of the solvent effect may be obtained by treatment of the titration data to evaluate the external (ΔH_{ext}) and internal (ΔH_{int}) contributions to ΔH in terms of Hepler's theory (*29*):

$$\Delta H = \Delta H_{int} + \Delta H_{ext} = \Delta H_{int} + \beta \Delta S \qquad [1]$$

According to this theory, ΔH_{int} arises from the enthalpy differences between the protonated and unprotonated forms of the molecules, including the inductive effects of the modifications at α-amino and carboxyl groups on imidazole basicity,

while ΔH_{ext} is associated with differences in solute–solvent interactions. The value of the proportionality constant, $\beta = 250$, was previously justified (*25*).

The ΔH_{int} values for N^{α}-AcHis, N^{α}-BocHis, and HO-desNH$_2$His (Table 4) are similar and negative, whereas the ΔH_{int} for HisOMe and HO-desNH$_2$HisOMe are significantly higher and positive, as expected when the α-amino or carboxyl group are blocked. The ΔH_{ext} values for N^{α}-AcHis and N^{α}-BocHis are not significantly different from zero, but for HO-desNH$_2$His ΔH_{ext} is higher and positive, suggesting a change of imidazole–solvent interaction due to the substitution of OH for NH_3^+. The significant negative ΔH_{ext} values of HisOMe and HO-desNH$_2$HisOMe indicate that esterification favors solvation of the imidazole group. Our results, therefore, indicate that solvent–solute interactions have to be taken into account to explain the smaller effect of the carboxylate than that of the α-ammonium group on the pK_a of histidine's imidazole group.

The methylation of the α-amino group (N^{α}-Me$_2$His and N^{α}-Me$_3$His) does not result in significant changes on either the pK_a values or the thermodynamic parameters for the titration of histidine's imidazole group. As the proposed $^{\alpha}NH_3^+ \ldots {}^{\pi}N\lessdot$ hydrogen bond (*12, 15, 16*) is not possible in these N^{α}-methylated histidine derivatives, the titration data do not support the presence of this bond in histidine. Further evidence against the hydrogen bond is provided by Fig. 2, in which the imidazole pK_a values of 4-substituted imidazoles are plotted against σ_I as defined by Charton (*30*). (The σ_I substitution constants were chosen for the plot of Fig. 2 because, as previously shown (*25*), the substituent inductive effects predominate over that of resonance in imidazole). It is seen (Fig. 2) that the data for histidine and HisOMe are within the 95% fiducial limits of the regression calculated for the series, which is given by

$$pK_a = 7.312 - 9.852\ \sigma_I \qquad [2]$$

Therefore, the interaction between the unprotonated N^{π} and α-ammonium groups is not strong enough to be reflected either by the thermodynamic parameters or by

TABLE 4

ΔH_{int} AND ΔH_{ext} FOR THE IMIDAZOLE GROUP TITRATION OF HISTIDINE AND DERIVATIVES

Compound	ΔpK_a[a]	ΔH_{int}[b,c] (kcal/mol)	ΔH_{ext}[b,c] (kcal/mol)
HO-desNH$_2$His	−1.29	−1.4 ± 0.4	1.5 ± 0.3
N^{α}-AcHis	−0.97	−1.2 ± 0.3	0.2 ± 0.2
N^{α}-BocHis	−1.04	−1.3 ± 0.3	0.5 ± 0.3
HisOMe	0.71	0.8 ± 0.3	−1.2 ± 0.2
HO-desNH$_2$HisOMe	0.58	0.6 ± 0.5	−1.2 ± 0.3

[a] Obtained by subtracting the pK_a value of the compound from that of imidazole group of histidine.

[b] Obtained from Eq. [1].

[c] Errors indicated are propagated standard deviations.

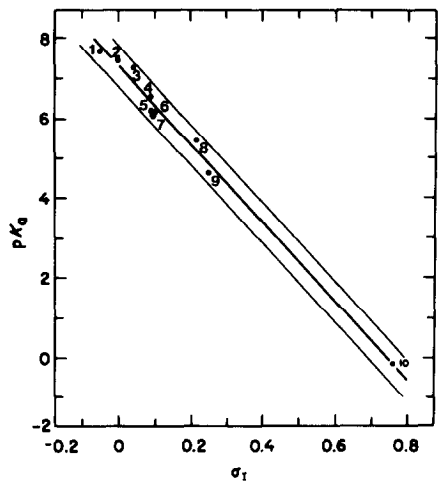
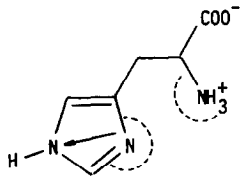


FIG. 2. pK_a of imidazolium groups of 4-substituted imidazoles vs σ_I as defined by Charton (*30*) in terms of pK_a values of the corresponding substituted acetic acid. The σ_I values for histidine and HisOMe were obtained from the γ-carboxyl group pK_a of glutamic acid and of α-methyl glutamate (*24*), respectively. (**1**) 4-methylimidazole (Ref. (*28*)); (**2**) imidazole (Ref. (*25*)); (**3**) 4-hydroxyethylimidazole (Ref. (*31*)); (**4**) 4-hydroxymethylimidazole (Ref. (*31*)); (**5**) histamine (Ref. (*21*)); (**6**) histidine; (**7**) 4-phenylimidazole (Ref. (*32*)); (**8**) HisOMe; (**9**) 4-aminomethylimidazole (Ref. (*33*)); (**10**) 4-nitroimidazole (Ref. (*34*)).

an anomalous behavior in the pK_a vs σ_I plot. A stronger interaction should be expected if hydrogen bond were present.

In spite of the evidence against the hydrogen bond, an interaction between the protonated amino group and N^{π} of the unprotonated imidazole ring in histidine is indicated by the interference of the amino group titration on the resonance of N^{π} as observed in ^{15}N nmr studies (*16, 18*) and also by the lower reactivity of the imidazole ring towards *p*-nitrophenylacetate in the presence of the charged α-amino group (*23*). Since the unprotonated imidazole group has a dipole moment whose orientation depends on the considered tautomer (*35*), an ion–dipole interaction, as shown in Scheme 3, is expected in histidine and should be preferred over the intramolecular hydrogen bond in polar solvents like water.

The imidazole pK_a values for N^{π}-MeHis and N^{τ}-MeHis show that N^{τ} is a stronger base than N^{π} and this is due both to enthalpy and entropy factors. As ΔH



SCHEME 3

is related mainly to N–H bond strength, the difference between the ΔH values of N^{τ}-MeHis and N^{π}-MeHis ΔH ($\Delta\Delta H$ = 1.6 kcal/mol) is the difference of proton-binding energy for the two imidazole-protonation sites. From molecular orbital studies two values were reported for this difference, 1.4 kcal/mol (*36*) and 0.1 kcal/mol (*37*), and from Raman spectra studies 1.0 kcal/mol when the α-amino group was unprotonated (*38*). The source of this N–H energy bond difference between the N^{π} and N^{τ} probably comes from the assimetry introduced by the substitution at position 4 of the imidazole ring (*39*).

The entropy component favored N^{π} deprotonation over that of N^{τ} and this effect should be related to less accessibility of N^{π} to solvent, as demonstrated by its very low reactivity towards *p*-nitrophenylacetate (*23*).

Amino groups (Table 3). Esterification of the histidine carboxyl group (HisOMe) decreases the α-amino pK_a by an entropy-controled process in which the values for thermodynamic parameters are similar to those of several other amino acids and their esters (*40*).

The α-amino group pK_a of $N^{\pi,\tau}$-Me_2His, in which the imidazole group has a permanent positive charge, is 1.3 units lower than that of histidine and this difference is also mainly reflected by the entropy contribution, since ΔS is 4.5 e.u. higher than that of histidine. A similar effect in ΔS was observed in α-amino groups of diamino acids (*41*), and this effect possibly occurs because the introduction of a charged group affects the change in solvent ordering which normally happens on deprotonation of the α-amino group.

The methylation of histidine's N^{π} (N^{π}-MeHis) reduces the α-amino group pK_a by 0.47 units, as is also observed in histidine by iodination of position 5 of the imidazole ring (*12*). In histamine, both the iodination at position 5 and the methylation at the π-nitrogen decrease the amino group pK_a by 0.5 units (*21, 42*). This behavior can be explained by admitting that both the iodination (by its negative inductive effect) and the methylation of N^{π} favor the N^{π}-H over the N^{τ}-H tautomer and this would invert the orientation of the imidazole ring dipole from $N^{\pi} \rightarrow N^{\tau}$ to $N^{\tau} \rightarrow N^{\pi}$, therefore lowering the N^{α} pK_a.

The α-amino group pK_a of N^{τ}-MeHis is not significantly different from that of histidine, due to compensation of the higher ΔH and ΔS values. A comparison of the values for the thermodynamic parameters of α-amino group titration of N^{π}-MeHis with those of N^{τ}-MeHis shows that the acidity of the N^{π}-MeHis ammonium group is favored by a $\Delta\Delta H$ = 1.3 kcal/mol and unfavored by a $\Delta\Delta S$ = 2.0 e.u. These data indicate an interaction of the α-ammonium group with the N^{π} of the unprotonated imidazole group in N^{τ}-MeHis, whose formation would involve a $\Delta G = -0.72$ kcal/mol, with an equilibrium constant of 1.0 for the bond formation. These data for N^{τ}-MeHis are compatible with the $^{\alpha}NH_3^+ \ldots {}^{\pi}N\lessdot$ hydrogen bond (*43*), which is favored in this particular histidine derivative by the permanent N^{τ}-H tautomeric form.

The α-amino group of N^{α}-Me_2His is a tertiary amine, with pK_a 0.22 units lower than that of histidine, and smaller ΔH (4.2 kcal/mol) and ΔS (13.1 e.u.) values. This is similar to what is observed with methylamine and trimethylamine (*24*), and is a general phenomenon for alkylamines, involving a well-defined water-solvation effect on determining their basicity (*44*).

CONCLUSIONS

An ion–dipole system better describes the interaction between the imidazole ring and the α-amino group of histidine. The $^{\alpha}NH_3^+ \ldots {}^{\pi}N\leqslant$ hydrogen bond does not seem to occur in histidine, but appears to be present in N^{τ}-MeHis.

Solvation effects on the imidazole ring have to be taken into account when the interactions between imidazole and neighboring groups are considered.

An enthalpy difference of 1.6 kcal/mol, favoring the N^{τ}-H over the N^{π}-H tautomer, indicates that the inductive substituent effect at position 4 of the imidazole ring is the main origin for preference of histidine for the N^{τ}-H tautomer, although steric hindrance by solvation of N^{π}-H tautomer could also contribute.

ACKNOWLEDGMENTS

This work was supported by grants from FINEP (B/76/81/384/00/00), FAPESP (82/0301-0), and CNPq (40.0327/81).

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