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Reactivity with *p*-Nitrophenyl Acetate and Interaction between the Amino and Imidazole Groups of Histidine and Related Compounds

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The study of the reaction of *p*-nitrophenyl acetate (PNPA) with histidine and certain derivatives showed that the species in which the amino group is unprotonated ($R(NH_2)Im$) react with second-order rate constants (k_2^{am}) that are higher than predicted by a Brønsted relation for a series of neutral amino acids. The reason for this behavior was investigated through an analysis of the kinetics of the reaction of PNPA with these compounds in order to assess the reactivities of the amino and imidazole groups in the two species $R(NH_3^+)Im$ and $R(NH_2)Im$. The rate constant for the reaction with the imidazole group (k_2^{im}) of N^{π}-methyl histidine agrees with the value predicted by a Brønsted relation obtained from a series of model imidazole compounds. N^{τ}-Methyl histidine, however, is unreactive, indicating that N^{τ} is the reactive nitrogen in the imidazole ring of histidine. The k_2^{im} values found for histidine, histidine methyl ester, and N^{α}-dimethyl histidine are lower than predicted by the Brønsted relation. This behavior was found to be due to low reactivity of the $R(NH_3^+)Im$ species, in contrast with the normal reactivity of $R(NH_2)Im$. The evidence presented suggests that the lower reactivity of $R(NH_3^+)Im$ is due to an ion–dipole interaction between the protonated amino group and the unprotonated imidazole ring, which displaces the tautomeric equilibrium toward the unreactive N^{τ}–H form. The higher reactivity of the imidazole group in the species $R(NH_2)Im$, relative to that in $R(NH_3^+)Im$, is responsible for the observed high k_2^{am} values for histidine, for histidine methyl ester, for N^{π}-methyl histidine, and for N^{α}-dimethyl histidine, in contrast with the normal k_2^{am} value found for N^{τ}-methyl histidine. The conclusions from this study of histidine and its derivatives support the proposal of an interaction between the protonated *N*-terminal amino group and the imidazole ring of His^6 in the octapeptide hormone angiotensin.

INTRODUCTION

In a previous study of the kinetics of reaction of *p*-nitrophenyl acetate (PNPA) with amino and imidazole groups of amino acids and peptides, the apparent second-order rate constants (k_2^{am}) for the reaction of PNPA with the amino groups of histidine and angiotensin (Asp–Arg–Val–Tyr–Ile–His–Pro–Phe) were found to be significantly higher than expected from the Brønsted correlation that fitted the data for the other compounds of the series (*1*). In the case of histidine, k_2^{am} was found to be 30.2 M^{-1} min^{-1}, well above the 95% fiducial limits (6.2–15.2 M^{-1} min^{-1}) predicted by the Brønsted relation. In the case of angiotensin and analogs,

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k_2^{am} was found to be 1.70 M^{-1} min^{-1}, compared to the 95% fiducial limits of 0.43 to 1.05 M^{-1} min^{-1}. The high k_2^{am} was interpreted, in the case of histidine, as a possible cooperative effect between the amino and imidazole groups, while no explanation was advanced for the case of angiotensin. Nevertheless, the electrostatic effect of the protonated *N*-terminal amino group of angiotensin on the proton dissociation of the imidazole group of its histidine side chain, has been interpreted as indication of proximity between these two groups (*2*). Therefore, a cooperative effect of the amino and imidazole groups of angiotensin might also be invoked to explain the high k_2^{am} observed in the reaction of that peptide with PNPA.

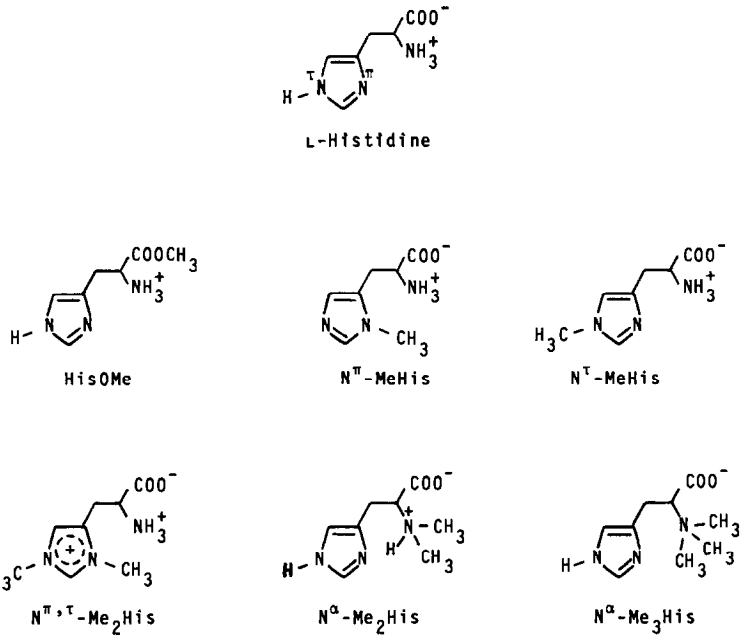
However, although general base catalysis of aminolysis of phenyl acetate has been described, this is not the case with PNPA, which is a more reactive compound with a better leaving group than phenyl acetate (*3*). Therefore, to better understand the apparently higher reactivity of the amino group in histidine and in angiotensin, we have studied the kinetics of the reaction of several histidine derivatives with PNPA, aiming at a detailed analysis of the contributions of the amino and imidazole groups.

The following compounds have been used in this study[1]: histidine, histidine methyl ester (HisOMe), N^{π}-methyl histidine (N^{π}-MeHis), N^{τ}-methyl histidine (N^{τ}-MeHis), $N^{\pi,\tau}$-dimethyl histidine ($N^{\pi,\tau}$-Me_2His), N^{α}-dimethyl histidine (N^{α}-Me_2His), and N^{α}-trimethyl histidine (N^{α}-Me_3His) (Scheme 1).

EXPERIMENTAL

All melting points were measured on a Monoscop VS Hans Bock micro hot stage apparatus and are uncorrected. Elemental analysis were performed at the Chemistry Institute of the University of São Paulo. Optical rotations were determined on a Bellingham & Stanley Pepol 60 spectropolarimeter, and ir spectra were recorded on a Perkin–Elmer Model 137 spectrophotometer. Proton magnetic resonance spectra were recorded with a Perkin–Elmer R-10 or a Varian XL-100-12, in D_2O solutions and using Me_4Si as the internal reference. Thin-layer chromatography (TLC) was done on 0.25-mm silica-gel G (E. Merck, Darmstadt) plates with the following solvent systems: A, *n*-butanol–acetic acid–water (4 : 1 : 1); B, *n*-butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1); C, *n*-butanol–pyridine–acetic acid–water (10 : 15 : 3 : 12); D, 96% ethanol–25% ammonium hydroxide (7 : 3); E, chloroform–methanol–25% ammonium hydroxide–water (20 : 22 : 5 : 5). Amino acid analyses were performed on a Beckman 120 C automatic amino acid analyzer, after hydrolysis of the peptide with 6 *N* HCl, in vacuum, at 100°C for 72 hr.

[1] To avoid the ambiguity in the nomenclature of the *N*-methyl histidines, due to the 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).



SCHEME 1

Calculations were done on a Varian 620/L-100 computer and with a Hewlett–Packard 9100A calculator with 9101A extended memory and 9125A plotter.

Materials

The following compounds were of the highest available grade from commercial sources: imidazole, 1-methyl imidazole, N^{α}-acetyl histidine, N^{π}-methyl histidine, N^{τ}-methyl histidine, and N^{α}-tBoc-N^{π}-methyl histidine from the Sigma Chemical Company; and histidine methyl ester and 4-phenyl imidazole from Aldrich.

The following compounds were synthesized as previously described: N^{α}-*tert*-butyloxycarbonyl histidine (*4*), N^{α}-dimethyl histidine and N^{α}-trimethyl histidine (*5*), N^{α}-acetyl histidine methyl ester (*6*), histidine amide (*7*), N^{α}-acetyl histidine amide (*8*), N^{α}-benzoyl histidine methyl ester (*9*), and hydroxydesamino histidine and its methyl ester (*10*).

$N^{\pi,\tau}$-Dimethyl histidine. Anhydrous N^{α}-benzoyl histidine methyl ester (15.5 g, 56.7 mmol) was refluxed in 280 ml anhydrous acetone and to the resulting solution were added 18.0 g (130 mmol) of anhydrous potassium carbonate and 15.0 ml (105 mmol) of methyl sulfate (d = 1.33 g/ml). After 32 hr of stirring in an oil bath at 80°C, the mixture was filtered, and the residue washed with 30 ml hot acetone. The solvent was evaporated and the resulting oil was hydrolyzed in 130 ml of 6.0 N hydrochloric acid at 100°C for 8 hr. The product was treated with 150 ml of ether and concentrated under reduced pressure, yielding an oil which was washed three times by dissolving in absolute ethanol and then adding anhydrous ether. The resulting oil (11.7 g) was dissolved in ethanol–water and left at −10°C for 2

days, yielding 6.0 g of a hygroscopic crystalline product. After recrystallization from ethanol–water, the product was purified in a column of Dowex 2-X8 (chloride form) equilibrated and eluted with water. After liophylization of the eluate the material was dried in vacuum at 100°C in an Abderhalden apparatus, yielding 3.2 g (11.0 mmol, 19.3%) of the product, which was homogeneous upon TLC in five solvent systems, with R_f 0.08 (A), 0.22 (B), 0.14 (C), 0.37 (D), and 0.32 (E); mp 222–224°C (*dec*); $(\alpha)_D^{26}$ +0.4° (*c* 1.68, 1 *N* HCl); ir (KBr, N_2 atmosphere) 3220–2270 (O-H, NH_3^+), 1740 (ν C=O), 1580 and 1510 (δ NH_3^+), 1202 (ν C=O) cm^{-1}; ^1H nmr (D_2O) 3.45 (d, 2H, J = 6.41 Hz), 3.87 (s, 3H) 3.90 (s, 3H), 4.25 (t, 1H, J = 6.41 Hz), 7.50 (s, 1H, Im-5-CH), 8.72 (s, 1H, Im-2-CH), *Anal.* Calcd for $C_8H_{19}N_3O_4Cl_2$: C, 32.89; H, 6.55; N, 14.38. Found: C, 32.68; H, 6.57; N, 14.23.

Hippuryl-histidine methyl ester. To 5.0 g (25.9 mmol) of hippuric acid hydrazide (*11*) suspended in 63.0 ml dimethylformamide, at −10°C, were added 17.6 ml of a 4 *N* solution of HCl in dioxane. The mixture was cooled to −25°C and 3.5 ml of isoamyl nitrite were added to the clear solution. After stirring for 90 min, 6.3 g (26 mmol) of histidine methyl ester dihydrochloride in dimethylformamide were added and the pH adjusted to 8.0 with triethylamine. The reaction mixture was left at −10°C for 5 days, filtered, and evaporated to dryness. The material was suspended in ethyl acetate, washed with 16% (w/v) potassium bicarbonate and with saturated NaCl solution, then dried overnight over Na_2SO_4. Crystallization from methanol–ether at −10°C yielded 4.0 g (12.1 mmol, 46.7%) of the product, which was homogeneous upon TLC in three systems, with R_f 0.48 (A), 0.65 (B), and 0.61 (C); mp 95–97°C; $(\alpha)_D^{29}$ −20.8° (*c* 1.73, 1 *N* HCl). Amino acid analysis indicated 86.4% peptide content and a glycine/histidine molar ratio of 1.06.

Kinetic measurements. The hydrolysis of PNPA was studied at several pH values, using the following buffer solutions: 0.05 *M* KH_2PO_4/K_2HPO_4 (pH 6.0–7.8); 0.1 *M* Tris–hydroxymethyl–amino methane (pH 7.8–8.8); 0.03 *M* $Na_2B_4O_7$ (pH 8.8–9.2). The ionic strengths of all solutions were adjusted to 0.20 by the addition of KCl. The compound under study was dissolved in the appropriate buffer and, after equilibration at 25.0°C (±0.1°C), the pH was adjusted to within 0.005 pH unit of the desired value, with 1 *N* KOH or HCl. The final concentration varied between 10^{-3} and 5×10^{-2} *M*. Two milliliters of the solutions were placed in a 1-cm quartz cell in the thermostated (25.0°C) compartment of a Shimadzu QV-50 spectrophotometer. The reaction was initiated by adding 0.025 ml of a solution of PNPA in ethanol. The final ethanol concentration in the reaction mixture was 1.2% (v/v) and the ester concentration was 10- to 100-fold smaller than that of the nucleophile. The appearance of *p*-nitrophenolate was measured at 400 nm for at least 80% of the reaction, when the pH was 7.8 or above, or for at least 40% of the reaction when the pH was lower. Under these conditions Lambert–Beer's law was found to apply to *p*-nitrophenolate, and pseudo-first-order kinetics was always observed. The first order rate constant (k_1) was obtained from

$$\ln(A_\infty - A_t)/(A_\infty - A_0) = -k_1 t \qquad [1]$$

where A_0, A_t, and A_∞ are, respectively, the absorbance at times zero (obtained by extrapolation), t, and at completion of the reaction. A_∞ was obtained from the

absorbance of a 10-fold dilution of the reaction mixture with 1 *N* KOH, by the equation

$$A_\infty = A_{max}(10^{pH-7.1}/(1 + 10^{pH-7.1})) \quad [2]$$

where A_{max} is the absorbance in 1 *N* KOH. The A_∞ values obtained in this way were frequently checked by absorbance measurements made in the reaction mixture after more than 15 half-lives, with very good agreement.

The second-order rate constants (k_2) were obtained from

$$k_2 = (k_1 - k_w)/c \quad [3]$$

where c is the nucleophile concentration and k_w is the rate constant for the reaction measured when $c = 0$. A blank for obtaining k_w was run simultaneously with each k_1 determination and the largest value obtained for k_w (at pH 9.2) did not exceed 50% of the corresponding k_1.

When only one nucleophilic group was present in the molecule, k_2 values were obtained in at least four pH's. When more than one nucleophilic group was present, data were obtained in at least 15 pH values in the range 6–9.7 (*1*). These data were fitted, by a least-squares method, to a multiple linear correlation of the form

$$k_2^{obs} = k_2^{im}\alpha^{im} + k_2^{am}\alpha^{am} \quad [4]$$

where k_2^{im} and k_2^{am} are the rate constants due to species $R(NH_3^+)Im$ and $R(NH_2)Im$, respectively, and α^{im} and α^{am} represent the fraction of the corresponding species present at each pH. The pK values for the imidazole and amino groups, determined by electrometric titrations, were used to calculate α^{im} and α^{am}.

Electrometric titrations were performed with a Radiometer Model 4 pH meter with a G202 C glass electrode and a K100 calomel electrode, calibrated with phosphate, phthalate, and borate buffers (*12*). Samples containing 0.05 mmol in 5.00 ml of 0.15 *M* KCl were placed in thermostated reaction flasks at 25.0°C (±0.1°C) and titrated by the addition of 1 *N* HCl or KOH from an "Agla" calibrated syringe driven by a "Burroughs Wellcome" micrometer. Not less than two independent titrations with at least 40 data points in the range of pH 2.5–10.5 were performed for each compound. The pK values were calculated by a least-squares method with an iterative computer program.

RESULTS AND DISCUSSION

We have confirmed that the k_2^{am} value for histidine is higher than that predicted by the Brønsted correlation previously obtained for α-branched amino acids (*1*). In Table 1 it is shown that k_2^{am} is also high for HisOMe and for N^π-MeHis, but not for N^τ-MeHis.

In Table 1 it is also shown that N^α-Me_2His has an appreciable k_2^{am} value, although its tertiary α-amino group should not interact with PNPA for steric reasons (*13*). This k_2^{am} value, associated with the species in which the tertiary amino group is deprotonated, can be attributed only to an increased reactivity of

TABLE 1

SECOND-ORDER RATE CONSTANTS (k_2^{am}) FOR THE REACTION OF PNPA WITH SPECIES R(NH_2)Im OF HISTIDINE AND SOME RELATED COMPOUNDS

Compound	pK_3	k_2^{am} (M^{-1} min^{-1})	
		Found[a]	Calcd[b]
Histidine	9.20	30.0 ± 0.3	6.2–15.2
HisOMe	7.36	13.3 ± 0.1	0.3– 0.7
N^{π}-MeHis	8.73	26.9 ± 0.7	2.8– 7.0
N^{τ}-MeHis	9.27	8.7 ± 0.1	7.0–17.1
N^{α}-Me_2His	8.98	16.9 ± 0.2	0[c]

[a] Obtained by least-squares fitting of experimental data to Eq. (4) (±SD).

[b] From a Brønsted relation for a series of α-branched amino acids (*1*): $\log k_2 = 0.726pK - 5.692$ (s 0.098).

[c] The tertiary amino group should not react for steric reasons.

the imidazole group in that species. If a similar effect is present in histidine, the abnormally high k_2^{am} found for this compound could be due to a summation of the amino group reaction with that due to the imidazole of species R(NH_2)Im of the ionization scheme 2.

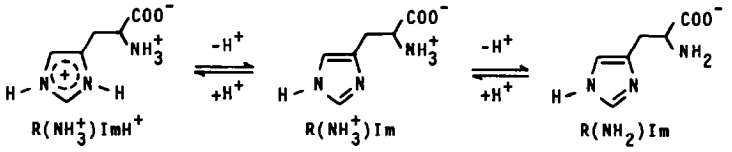
To verify this hypothesis we have analyzed the kinetics of reaction of PNPA with several histidine derivatives, attempting to separate the contributions from the amino and imidazole groups in species R(NH_2)Im.

Reactivity of the imidazole group. The Brønsted plot for a series of model compounds in which the imidazole group is the only nucleophile reacting with PNPA (Fig. 1) yielded the correlation

$$\log k_2^{im} = 0.569pK - 2.755 \qquad (r = 0.938, \quad s = 0.049) \qquad [5]$$

where r is the correlation coefficient and s is the standard error of the estimate. The k_2^{im} value observed with N^{α}-Me_3His, where the positive charge is permanent, was abnormally low, and was not included in the obtention of Eq. [5].

The k_2^{im} values for histidine and other imidazole derivatives in which an amino group is also present are shown in Table 2. It is striking that the reactivity of N^{τ}-MeHis was negligible, while the k_2^{im} of N^{π}-MeHis was within the range predicted by Eq. [5]. This finding indicates that PNPA reacts with the *tele* nitrogen of the imidazole group, but not with the *pros* nitrogen, probably because the latter is



SCHEME 2

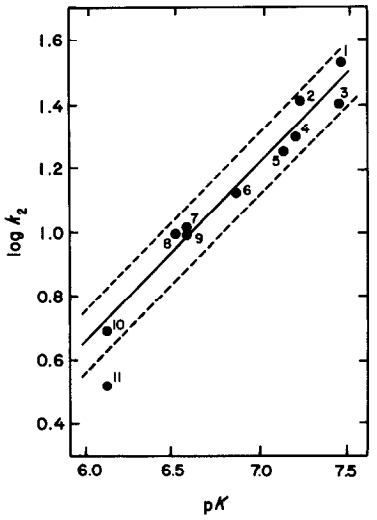


FIG. 1. Brønsted plot for the reaction of PNPA with a series of imidazole compounds: 1, imidazole; 2, 1-methyl imidazole; 3, hydroxydesamino histidine; 4, *t*-butyloxycarbonyl histidine; 5, acetyl histidine; 6, hydroxydesamino histidine methyl ester; 7, acetyl histidine methyl ester; 8, hyppuryl histidine methyl ester; 9, acetyl histidine amide; 10, 4-phenyl imidazole; 11, N^{α}-trimethyl histidine. —, Eq. (5), obtained by the least squares method; ---, 95% fiducial limits.

hindered by the 2-(β-amino,β-carboxy)-ethyl substituent at position 4 of the ring. The *tele* nitrogen, when deprotonated, would then be responsible for the reactivity of the imidazole group in histidine. Accordingly, the N^{π}–H tautomer would be reactive and the N^{τ}–H unreactive (Scheme 3).

Titration results obtained with histidine and some of its derivatives (*14*) show that in species $R(NH_3^+)Im$ the preferred tautomer is the N^{τ}–H, while in species

TABLE 2

SECOND-ORDER RATE CONSTANTS (k_2^{im}) FOR THE REACTION OF PNPA WITH SPECIES $R(NH_3^+)Im$ OF HISTIDINE AND SOME RELATED COMPOUNDS

Compound	pK_2	k_2^{im} (M^{-1} min^{-1}) Found[a]	Calcd[b]
Histidine	6.14	3.0 ± 0.1	4.2– 6.7
HisOMe	5.43	0.8 ± 0.3	1.6– 2.6
N^{π}-MeHis	6.61	9.9 ± 0.6	7.9–12.5
N^{τ}-MeHis	5.99	0.1 ± 0.2	3.5– 5.5
N^{α}-Me_2His	6.15	3.3 ± 0.1	4.3– 6.8

[a] Obtained by least-squares fitting of experimental data to Eq. (4) (±SD).

[b] The ranges indicate the 95% fiducial limits calculated from Eq. (5).

$R(NH_2)Im$ both N^{τ}–H and N^{π}–H forms are about equally populated. Therefore, the imidazole group would be expected to be more reactive in $R(NH_2)Im$ than in $R(NH_3^+)Im$. This is in agreement with the low k_2^{im} values found for N^{α}-Me_3His, where $R(NH_3^+)Im$ is the only reactive species, and for histidine and HisOMe (Table 2), in which k_2^{im} values represent mainly the reactivity of the $R(NH_3^+)Im$ species.

To further investigate this hypothesis we have isolated the second-order rate constants for the reaction of PNPA with the amino ($k_2^{am_0}$) and with the imidazole ($k_2^{im_0}$) groups of species $R(NH_2)Im$. For this purpose the observed k_2^{am} values obtained from Eq. [4] were taken as the sum of $k_2^{am_0}$ and $k_2^{im_0}$ as shown in

$$k_2^{obs} = k_2^{im}\alpha^{im} + (k_2^{am_0} + k_2^{im_0})\alpha^{am} \qquad [6]$$

where α^{im} and α^{am} are, respectively, the fractions of species $R(NH_3^+)Im$ and $R(NH_2)Im$ present in each pH.

Values of $k_2^{am_0}$ for the different compounds were calculated by means of the Brønsted equation previously derived from data obtained with a large number of amino acids (*1*):

$$\log k_2^{am_0} = 0.726pK_3 - 5.692 \qquad (r = 0.957, \quad s = 0.098) \qquad [7]$$

where pK_3 is the pK of the amino group. Values for $k_2^{im_0}$ were estimated by subtracting $k_2^{am_0}$ from k_2^{am}. In order to see whether the $k_2^{im_0}$ values thus obtained for the different compounds would fit the Brønsted relation for imidazole groups (Eq. [5]), it was necessary to estimate the basicity of the imidazole group in the respective $R(NH_2)Im$ species. For this purpose, we have obtained imidazole pK values from electrometric titrations of appropriate model compounds. N^{α}-Acetyl histidine (N^{α}-Ac-His) was used as a model for histidine and for N^{α}-Me_2His. The model used for HisOMe was N^{α}-acetyl histidine methyl ester (N^{α}-Ac-His-OMe) and for N^{π}-MeHis we used N^{α}-*tert*-butyloxycarbonyl-N^{π}-methyl histidine (N^{α}-tBoc-N^{π}-MeHis). The pK values for these model compounds are shown in Table 3. In this table it is also shown that the $k_2^{im_0}$ values for histidine and its derivatives were within the limits predicted by the Brønsted relation obtained with simple imidazole compounds (Eq. [5]). Therefore, the reactivity of the imidazole group of histidine and its derivatives is normal in the absence of amino group protonation. This contrasts with the low k_2^{im} values found for the $R(NH_3^+)Im$ species of these compounds, with an exception in the case of N^{π}-MeHis (Table 2).

These results agree with the propositions that the imidazole group is more reactive in species $R(NH_2)Im$ than in $R(NH_3^+)Im$ and that N^{τ} is the reactive nitrogen in the ring.

N^{τ}-H tautomer N^{π}-H tautomer

SCHEME 3

TABLE 3

SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF PNPA WITH THE IMIDAZOLE GROUP OF SPECIES $R(NH_2)Im$

Compound	Model	pK	$k_2^{im_0}$ Found	$k_2^{im_0}$ Calcd[a]
Histidine	N^{α}-Ac-His	7.11	20 ± 4	16–25
HisOMe	N^{α}-Ac-HisOMe	6.56	12.0 ± 0.6	8–12
N^{π}-MeHis	N^{α}-*t*-Boc-N^{π}-MeHis	7.28	22 ± 2	20–31
N^{α}-Me_2His	N^{α}-Ac-His	7.11	16.9 ± 0.2	16–25

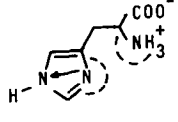
[a] 95% fiducial limits, estimated from the pK values of model compounds by means of Eq. (5).

A positive charge in the amino group thus appears to exert a significant effect on the reactivity of the imidazole ring toward PNPA. On the other hand, a positive charge on the imidazole ring did not affect amino group reactivity. This was shown by the finding, for $N^{\pi,\tau}$-Me_2His (pK 7.85), of $k_2^{am} = 0.8 \pm 0.1$, which is within the limits (0.6–1.6) predicted by Eq. [7].

An interaction between the protonated amino group and N^{π} of the unprotonated imidazole ring in histidine is supported by several pieces of evidence. Thus, methylation at N^{π} lowers histidine pK_3 by 0.47 pH units, while N^{τ}-methylation does not greatly affect the amino group ionization (compare pK_3 for N^{π}-MeHis and N^{τ}-MeHis in Table 1). A decrease of 0.5 pH units in histidine pK_3 also occurs upon iodination of position 5 of the imidazole ring (*15*). In histamine, both the methylation of the *pros* nitrogen (*16*) and iodination at position 5 of the ring (*17*) also result in the lowering of pK_3 by 0.5 pH units.

We believe that the above-described behavior is caused by the fact that both the iodination at position 5 of the ring and the methylation of N^{π} would favor the N^{π}–H over the N^{τ}–H tautomer, which is normally prevalent in the unprotonated imidazole group of histidine. Thus both the methylation and the iodination would invert the orientation of the imidazole ring dipole (see Scheme 4) from $N^{\pi} \rightarrow N^{\tau}$ to $N^{\tau} \rightarrow N^{\pi}$, resulting in the observed effect on the basicity of the amino group.

Concerning the nature of the interaction between the protonated amino group and the N^{π} of the unprotonated imidazole ring in histidine, a hydrogen bond has been proposed on the basis of titrations of iodinated derivatives (*15*) as well as nmr data (*18, 19*). Our results, however, may be explained on the basis of only the ion–dipole interaction discussed above. Indeed, N^{α}-Me_3His, in which the proposed hydrogen bond is impossible, has an abnormally low k_2^{im} (Fig. 1), similarly to what is observed for histidine (Table 2). This indicates that the observed effect



SCHEME 4

of the protonated amino group upon the reactivity of the imidazole ring is due to the positive charge on that group rather than on a possible $\geq N^{\pi} \cdots H_3{}^{+}N^{\alpha}$-hydrogen bond. It should be also noted that the imidazole pK of N^{α}-Me_3His (6.12) does not significantly differ from that of histidine (6.14). This indicates that any effect of the protonated amino group on the ionization of the imidazole group of histidine may be ascribed to an electrostatic interaction.

In view of our present hypothesis, the previous finding (*1*) that $k_2{}^{am}$ for angiotensin is higher than predicted by Eq. [7] is in agreement with the titration data, which suggested an electrostatic interaction between the *N*-terminal amino and His^6 imidazole side chain in that peptide (*2*). Conversely, the analog (Pro^3, Pro^5)-angiotensin, for which that interaction was not indicated by titration (*2*) or nmr data (*20*), had a normal $k_2{}^{am}$ value (*1*), as predicted by Eq. [7]. An interaction between these amino and imidazole groups in angiotensin, similar to that postulated for histidine, is further supported by the finding that the N^{τ}–H/N^{π}–H tautomeric ratio in angiotensin is about 4, which is the value also found for histidine (*14, 21*).

Modulation of imidazole reactivity by the proximity of other groups may be important for the biological activities of some peptides and proteins. This is found, for example, in serine proteases, where the reactivity of the imidazole group of His^{52} in the active site is modulated by the carboxyl group of Asp^{102}. This interaction would favor the N^{τ}–H tautomer, increasing the reactivity at the *tele* nitrogen (*22*). The compounds studied in this paper, as well as the angiotensin peptides, may be useful as models for such interactions in more complex molecules.

CONCLUSIONS

The reaction of PNPA with the imidazole group of histidine occurs at the *tele* nitrogen, the *pros* nitrogen being unreactive. When the amino group is protonated the ion–dipole interaction with the imidazole group favors the N^{τ}–H tautomer, leading to a smaller reactivity of the $R(NH_3{}^{+})Im$ species relative to that of $R(NH_2)Im$. As a result, histidine, and its derivatives in which the ion–dipole interaction occurs, have lower $k_2{}^{im}$ values than those predicted by the Brønsted relation for a series of simple imidazole compounds. Conversely, $k_2{}^{am}$ values for these compounds are higher than predicted by the Brønsted relation for simple amino acids, because it includes a contribution from the increased imidazole reactivity in the species $R(NH_2)Im$ relative to that in $R(NH_3{}^{+})Im$.

The abnormally high $k_2{}^{am}$ found for angiotensin, as compared to the normal value for the analog (Pro^3, Pro^5)-angiotensin, suggests an interaction between the *N*-terminal amino and His^6 imidazole groups of that hormone. This is further supported by the similarity of the imidazole tautomeric ratios in angiotensin and histidine, as well as by electrometric titration and nmr data.

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REFERENCES

1. L. JULIANO, P. BOSCHCOV, AND A. C. M. PAIVA, *Biochemistry* **13,** 4263 (1974).
2. L. JULIANO AND A. C. M. PAIVA, *Biochemistry* **13,** 2445 (1974).
3. W. P. JENCKS AND J. CARRIUOLO, *J. Amer. Chem. Soc.* **82,** 675 (1960).
4. G. FLOURET, R. MORGAN, R. GENDRICH, J. WILBER, AND M. SEIDEL, *J. Med. Chem.* **16,** 1137 (1973).
5. V. N. REINHOLD, Y. ISHIKAWA, AND D. B. MELVILLE, *J. Med. Chem.* **11,** 258 (1968).
6. W. NAGAI, K. L. KIRK, AND L. A. COHEN, *J. Org. Chem.* **38,** 1971 (1973).
7. L. LEVINTOW, V. E. PRICE, AND J. P. GREENSTEIN, *J. Biol. Chem.* **184,** 55 (1950).
8. A. NEUBERGER, *Biochem. J.* **32,** 1452 (1938).
9. O. GERNGROSS, *Z. Physiol. Chem.* **108,** 50 (1919).
10. J. WAGNER AND G. RAUSCH, *Z. Anal. Chem.* **194,** 350 (1963).
11. T. CURTIUS, *Ber.* **35,** 3226 (1902).
12. R. G. BATES, "Electrometric pH Determinations," p. 118. Wiley, New York, 1954.
13. W. P. JENCKS AND J. CARRIUOLO, *J. Amer. Chem. Soc.* **82,** 1778 (1960).
14. A. C. M. PAIVA, L. JULIANO, AND P. BOSCHCOV, *J. Amer. Chem. Soc.* **98,** 7645 (1976).
15. K. J. BRUNINGS, *J. Amer. Chem. Soc.* **69,** 205 (1947).
16. C. R. GANELLIN, *J. Pharm. Pharmacol.* **25,** 787 (1973).
17. T. B. PAIVA, M. TOMINAGA, AND A. C. M. PAIVA, *J. Med. Chem.* **13,** 689 (1970).
18. F. BLOMBERG, W. MAURER, AND H. RÜTERJANS, *J. Amer. Chem. Soc.* **99,** 8149 (1977).
19. R. E. LONDON, *J. Chem. Soc. Chem. Commun.* 1070 (1978).
20. R. DESLAURIERS, R. A. KOMOROSKI, J. C. LEVY, A. C. M. PAIVA, AND I. C. P. SMITH, *FEBS Lett.* **62,** 50 (1976).
21. R. DESLAURIERS, A. C. M. PAIVA, J. SCHAUMBURG, AND I. C. P. SMITH, *Biochemistry* **14,** 878 (1975).
22. R. M. STROUD, M. KRIEGER, R. E. KOEPPE II, A. A. KOSSIAKOFF, AND J. L. CHAMBERS, in "Proteases and Biological Control" (E. Reich, D. B. Rifikin, and E. Shaw, Eds.), Vol. 2, p. 13. Cold Spring Harbor Symposia, Cold Spring Harbor, N.Y., 1975.