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Brønsted Relation for Hydrogen→Deuterium Exchange Reactions of Imidazole-bearing Molecules with Amino Groups

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The base-catalyzed hydrogen→deuterium exchange rates of the imidazole C-2 protons were measured for the imidazole-bearing molecules with amino groups ($N^2H_2 \cdot Im$) in ^2H_2O at various pH. From the pH dependences of the pseudo-first-order rate constants, the second-order rate constants were obtained for the $N^2H_3^+ \cdot Im^2H^+$ species and for the equilibrium mixtures of the $N^2H_2 \cdot Im^2H^+$ and $N^2H_3^+ \cdot Im$ species. For both the $N^2H_3^+ \cdot Im^2H^+$ and $N^2H_2 \cdot Im^2H^+$ species, the second-order rate constants and microscopic dissociation constants of the imidazolium rings were found to satisfy the Brønsted relation, with concomitant estimation of the abundance ratio of the $N^2H_2 \cdot Im^2H^+$ and $N^2H_3^+ \cdot Im$ species. This abundance ratio was compared with that estimated from the pH dependence of proton chemical shifts.

The C-2 proton of imidazole ring is known to exchange with the hydrogen isotopes (deuterium or tritium) under mild condition by a base-catalyzed scheme.[1-3] For the hydrogen→tritium exchange reactions of imidazole-bearing molecules without amino groups, Minamino *et al.* have found that the second-order rate constants k_b and pK_a's of imidazole rings satisfy the Brønsted relation:

$$\log k_b = \alpha pK_a + \beta, \tag{1}$$

where α and β are the constants for this type of relation.[4]

For the determination of pK_a values the nuclear magnetic resonance (NMR) spectroscopy is useful. The second-order rate constants (k_b) for hydrogen→deuterium exchange reactions may also be measured by NMR, without radioisotope manipulations. Here it may be noted that (1) the rate constant k_b for hydrogen→deuterium exchange reaction may be different from that for hydrogen→tritium exchange reaction; (2) the pK_a value of imidazole ring in ^2H_2O solution is different from that in H_2O solution; (3) the correct k_b values may not be obtained by a simple relation (see Eq. 2) if the imidazole ring is in proximity to other positively-charged ionizable groups with pK_a values close to that of the imidazole ring.

In the present study a novel method for the Brønsted plots (log k_b *versus* pK_a) for hydrogen→deuterium exchange reactions of imidazole-bearing molecules with amino groups has been worked out. These Brønsted plots together with those for imidazole-bearing molecules with no amino groups give a single straight line, and the validity of the Brønsted relation has been confirmed.

Experimental

The imidazole-bearing molecules used in the present study are *trans*-urocanic acid, *N*-acetylhistidine methylamide, *N*-acetylhistidine, and imidazole-4-acetic acid with no amino groups, and histidine methylamide, histidylglycine, histidinol, histidine and histamine with amino groups. 270-MHz proton NMR spectra were recorded on a Bruker WH-270 spectrometer. pH values (direct pH-meter readings)[5] were measured by a Radiometer PHM-26 pH meter. pK_a values were obtained by the nonlinear least square analyses of the pH dependences of proton chemical shifts. The hydrogen→deuterium exchange reaction was carried out in ^2H_2O solution (0.2 M NaCl, 1 M=1 mol dm^{-3}) at 37 °C directly in the 5-mm glass NMR tubes. The intensity decay of the C-2 proton resonance was measured relative to the intensity of C-4 proton resonance, and the observed pseudo-first-order rate constant k_{obsd} were obtained from its semi-log plots.

Results and Discussion

Brønsted Plots for Imidazole-bearing Molecules with No Amino Groups. For the imidazole-bearing molecules with no amino groups, it was sufficient to do hydrogen→deuterium exchange experiments at a given pH (9—11). The second-order rate constant k_b was obtained from k_{obsd} by using the following equation:

$$k_{obsd} = \{k_\phi K_a/[^2H^+]\}/\{1+K_a/[^2H^+]\} \tag{2}$$

$$k_\phi = k_b K_w/K_a, \tag{3}$$

where K_a is the dissociation constant of the imidazolium ring and K_w is the ion product of H_2O ($10^{-13.6}$ at 37 °C). The results are shown in Table 1 and their Brønsted plots in Fig. 1. As clearly seen in Fig. 1, log k_b and pK_a values are correlated by a single straight line and thus satisfy the Brønsted relation ($\alpha=-0.76$ and β=6.7 in Eq. 1).[6]

Brønsted Plots for Imidazole-bearing Molecules with Amino Groups. For the imidazole-bearing molecules with amino groups (denoted as $N^2H_2 \cdot Im$), the time course of exchange reaction was followed at eight pH's in the range 3—12. For example, the pH dependence of k_{obsd} for histidine is shown in Fig. 2. Exchange reaction does not occur at the acidic pH indicating that acid (or ^2H_2O)-catalyzed exchange mechanism can be neglected. This pH dependence curve exhibits two inflections at pH's each corresponding to the pK_a's of the imidazolium ring and the amino group, as previously reported.[7,8]

For this case k_{obsd} is written as,

$$k_{obsd} = \frac{k_{\phi 1}K_{a1}/[^2H^+]+k_{\phi 2}K_{a1}K_{a2}/[^2H^+]^2}{1+K_{a1}/[^2H^+]+K_{a1}K_{a2}/[^2H^+]^2} \tag{4}$$

$$k_{\phi 1} = k_{b1}K_w/K_{a1} \tag{5}$$

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Table 1. Dissociation constant (K_a in M) and second-order rate constant (k_b in $s^{-1} M^{-1}$) for imidazole-bearing molecules without amino groups

	Molecule	pK_a	log k_b
A	*trans*-Urocanic acid	5.82	2.26
B	*N*-Acetylhistidine methylamide	6.45	1.79
C	*N*-Acetylhistidine	7.07	1.32
D	Imidazole acetic acid	7.55	0.93

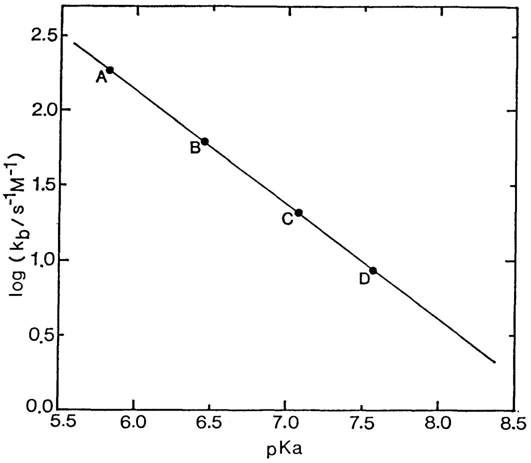

Fig. 1. Brønsted plots (k_b *versus* pK_a) for hydrogen→deuterium exchange reactions of imidazole-bearing molecules without amino groups.
For the notations A—D, see Table 1.

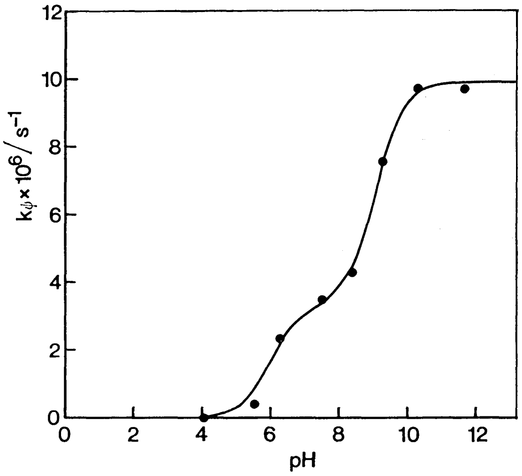

Fig. 2. pH dependence of the observed rate constant k_{obsd} for hydrogen→deuterium exchange reaction of histidine.

$$k_{\phi 2} = \rho k_{b2} K_w / K_{a2} \quad (6)$$

$$\rho/(1-\rho) = [N^2H_2\cdot Im^2H^+]/[N^2H_3^+\cdot Im], \quad (7)$$

where K_{a1} and K_{a2} are the first and second dissociation constants respectively, of $N^2H_3^+\cdot Im^2H^+$, k_{b1} and k_{b2} are the second-order rate constants for the reaction of O^2H^- ion with $N^2H_3^+\cdot Im^2H^+$ and $N^2H_2\cdot Im^2H^+$ species respectively. Here it should be noted that the C_α–C_β rotamer populations of histidine compounds are not significantly perturbed by the ionization of the imidazole ring [J. Kobayashi, T. Higashijima, S. Sekido, and T. Miyazawa, personal communication]. By the use of Eqs. 4—7, the values of $k_{\phi 1}$ and $k_{\phi 2}$ were obtained by applying the nonlinear least-square method to the pH-dependence curves of k_{obsd}. These $k_{\phi 1}$ and $k_{\phi 2}$ were then used for calculating k_{b1} and ρk_{b2} (in $s^{-1} M^{-1}$) as shown in Table 2.

Table 2. Dissociation constants (K_{a1} and K_{a2} in M) and second-order rate constants (k_{b1} and k_{b2} in $s^{-1} M^{-1}$) for imidazole-amino bearing molecules

	Molecule	pK_{a1}	log k_{b1}	pK_{a2}	log ρk_{b2}
A	Histidine methylamide	5.45	2.44	7.42	1.02
B	Histidylglycine	5.84	2.10	7.62	0.85
C	Histidinol	5.90	2.10	8.90	−0.32
D	Histidine	6.06	2.08	9.16	−0.56
E	Histamine	6.18	2.07	9.92	−1.27

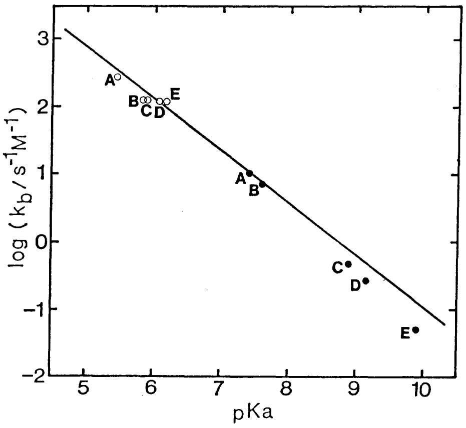

Fig. 3. Brønsted plots for hydrogen→deuterium exchange reactions of imidazole-amino-bearing molecules; ○ for k_{b1} *versus* K_{a1}; ● for ρk_{b2} *versus* K_{a2}.
For the notations A—E, see Table 2.

For this case the Brønsted plots of log k_{b1} and pK_{a1} (open circles in Fig. 3) show the tendency of slight deviations downward from the Brønsted line. The Brønsted plots of log ρk_{b2} and pK_{a2} (closed circles in Fig. 3) lie also downward from the line.

Correction of Brønsted Plots Taking Account of Microscopic Equilibrium. The deviation from the Brønsted relation of the plots for imidazole-bearing molecules with amino groups (Fig. 3) indicates the necessity of taking into account the microscopic equilibrium. That is, the second-order rate constant k_{b1} for the $N^2H_3^+\cdot Im^2H^+$ species should be correlated with the dissociation constant $K_1=(1-\rho)K_{a1}$ for the microscopic equilibrium $N^2H_3^+\cdot Im^2H \rightleftarrows N^2H_3^+\cdot Im + {}^2H^+$. The difference between pK_1 and pK_{a1} is equal to log $[1/(1-\rho)]$. On the other hand, the second-order rate constant k_{b2} for the $N^2H_2\cdot Im^2H^+$ species is to be correlated with the dissociation constant $K_2=K_{a2}/\rho$ for the microscopic equilibrium $N^2H_2\cdot Im^2H^+ \rightleftarrows N^2H_2\cdot Im + {}^2H^+$. The differences between log ρk_{b2} and log k_{b2} and between pK_2 and pK_{a2} are both equal to log $(1/\rho)$. In

TABLE 3. DISSOCIATION CONSTANTS (K_1 AND K_2 IN M), SECOND-ORDER RATE CONSTANTS (k_{b1} AND k_{b2} IN s^{-1} M^{-1}), AND EQUILIBRIUM CONSTANT (ρ) AND ITS STANDARD DEVIATION (σ) FOR IMIDAZOLE-AMINO-BEARING MOLECULES

Molecule		pK_1	$\log k_{b1}$	pK_2	$\log k_{b2}$	ρ (σ)
A	Histidine methylamide	5.71	2.44	7.07	1.37	0.45 (0.08)
B	Histidylglycine	6.10	2.10	7.27	1.20	0.45 (0.04)
C	Histidinol	5.97	2.10	8.08	0.50	0.15 (0.04)
D	Histidine	6.11	2.08	8.16	0.44	0.10 (0.02)
E	Histamine	6.19	2.07	8.22	0.43	0.02 (0.01)

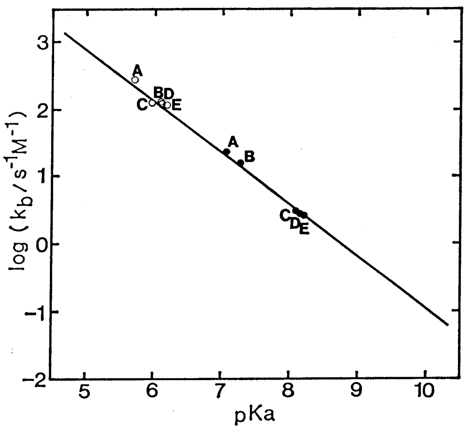

Fig. 4. Brønsted plots for hydrogen→deuterium exchange reactions of imidazole-amino-bearing molecules; ○ for k_{b1} *versus* K_1; ● for k_{b2} *versus* K_2. For the notations A—E, see Table 3.

fact, after adjusting the ratio ρ in the range 0—1, the Brønsted plots for k_{1b} (open circles in Fig. 4) and for k_{b2} (closed circles in Fig. 4) simultaneously lie close to the straight line, on the assumption that the imidazole-bearing molecules with amino groups follow the same Brønsted relation as obtained for the imidazole-bearing molecules with no amino groups. Thus, the values of ρ, pK_1, pK_2, and $\log k_{b2}$ were determined, as shown in Table 3, for the imidazole-bearing molecules with amino groups. The standard deviation for a pair of corrected Brønsted plots (pK_1, $\log k_{b1}$) and (pK_2, $\log k_{b2}$) is now smaller than that for a pair of uncorrected Brønsted plots (pK_{a1}, $\log k_{b1}$) and (pK_{a2}, $\log \rho k_{b2}$); the standard deviation is reduced from 0.12 to 0.091 for histidine methylamide, 0.28 to 0.08 for histidinol, 0.30 to 0.06 for histidine, and 0.44 to 0.08 for histamine.

It may be remarked that the second-order rate constant k_{b1} for the $N^2H_3^+ \cdot Im^2H^+$ species are 10—60 times as large as those (k_{b2}) for the $N^2H_2 \cdot Im^2H^+$ species, indicating that the reactivity of the imidazolium ring with O^2H^- ion is enhanced by the presence of the $N^2H_3^+$ groups in the proximity. Thus, as long as the base-catalyzed scheme is valid, the Brønsted relation applies to the second-order rate constants k_{b1} and k_{b2} with the concomitant estimation of the fractional population ρ of $[N^2H_2 \cdot Im^2H^+]$.

Comparison of ρ Estimated from the Brønsted-plot Correction with That from the pH Dependence of Proton Chemical Shifts. In the base catalyzed hydrogen→deuterium exchange reaction, O^2H^- ions react only with the species whose imidazole rings are protonated *i.e.* $N^2H_3^+ \cdot Im^2H^+$ and $N^2H_2 \cdot Im^2H^+$. This is the reason why we can estimate the microscopic equilibrium constant ρ (the fractional population of $[N^2H_2 \cdot Im^2H^+]$). ρ may be also determined from the pH dependences of proton chemical shifts if some reasonable assumptions are made.[9] One possible assumption is that the increase in the chemical shift upon protonation is equal to that of *N*-acetylhistidine methylamide[10] whether or not the neighboring amino group is protonated. This assumption allows us to calculate the chemical shifts for $N^2H_2 \cdot Im^2H^+$ (δ_{0+}) and $N^2H_3^+ \cdot Im$ (δ_{+0}) from the first and third limiting chemical shifts for $N^2H_2 \cdot Im$ (δ_{00}) and $N^2H^+{}_3 \cdot Im^2H^+$ (δ_{++}). Then ρ may be determined by using the relation $\rho = (\delta_+ - \delta_{+0})/(\delta_{0+} - \delta_{+0})$, where δ_+ is the second limiting shift obtained from the least-square fitting of the pH titration curve with the simple sum of two Henderson-Hasselbalch functions. Thus values of ρ were calculated for the five imidazole-bearing molecules with amino groups from the pH dependences of their C-2 and C-4 proton chemical shifts, and are listed in Table 4.

TABLE 4. EQUILIBRIUM CONSTANT (ρ) CALCULATED FROM THE pH DEPENDENCES OF C-2 AND C-4 PROTON CHEMICAL SHIFTS FOR IMIDAZOLE-AMINO-BEARING MOLECULES

Molecule		ρ (C-2 proton)	ρ (C-4 proton)
A	Histidine methylamide	0.17	0.14
B	Histidylglycine	0.26	0.15
C	Histidinol	0.02	0.02
D	Histidine	0.03	0.06
E	Histamine	0.03	0.07

Now the values of ρ obtained by the two independent methods, the Brønsted-plot correction procedure (Table 3) and the analyses of the pH dependence of proton chemical shifts (Table 4), may be compared. Although ρ's in Table 4 are smaller than those in Table 3, the trend in ρ's among the five compounds is common to Table 3 and Table 4; histidine methylamide and histidinol have larger ρ's than the other three compounds. The differences in ρ's between Table 3 and Table 4 are probably due to that (1) the assumption used for calculating ρ in Table 4 is too simple because ρ's for C-2 proton chemical shifts differ from those for C-4 proton chemical shifts; (2) the accuracy of ρ in Table 3 is not sufficient for the critical discussion at present.

In conclusion, if an ionizable group with a pK_a

of 6—12 is in proximity to the imidazole ring, the pH dependence of the pseudo-first-order rate constant should be followed and be analyzed on the basis of microscopic equilibrium. Then the reactivity of the imidazole-ring C-2 proton may be compared on the Brønsted plots with those of imidazole-bearing molecules without such ionizable groups. Thus the validity of the Brønsted relation for the imidazole-bearing molecules even with amino groups was confirmed in the present study. This Brønsted relation will hopefully provide a basis for studying the perturbation on the reactivity of histidine residues in proteins.

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5) Generally p^2H is expressed as $p^2H = pH$(meter reading) $+0.4 - \Delta pK_a$, where ΔpK_a is an isotope effect on the acidity constants of ionizable groups (A. Bundi and K. Wüthrich, *Biopolymers*, **18**, 285 (1979)) and is in the range *ca.* 0.5—0.7. However our discussion in the present study is valid without such an isotope correction, for we adopted pH(meter reading) in *all* the measurement and data interpretation *e.g.* in the measurements of pH dependence of exchange rate constants and proton chemical shifts.

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