Hydrogen-deuterium exchange kinetics of the C-2 protons of imidazole and histidine compounds [20]

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Hydrogen–Deuterium Exchange Kinetics of the C-2 Protons of Imidazole and Histidine Compounds[1]

Sir:

The kinetics of the deuteration at the 2 position in imidazole and some substituted imidazoles has been studied by various workers.[2–5] The mechanism proposed for the reaction involves the interaction of the protonated form of the imidazole with OD^- or D_2O, with replacement of the proton at the 2 position by a negative charge to produce an ylide (slow step). The second, fast step, involves reaction of the ylide with D_2O, with substitution of deuterium at the 2 position.[5] We have been concerned with the determination of the pK values[6–8] and the kinetics of the deuteration of histidine residues in proteins.[9] In this communication we report on the kinetics of the deuteration of various substituted imidazole and histidine compounds, which serve as suitable model compounds for the exchange behavior in proteins.

The purities of the various model compounds shown in Table I were checked by pmr spectroscopy. The

Table I

Compound	Apparent dissociation constants[a] pK_1	pK_2	pK_3	$k_1 \times 10^{-3}$, l. mol^{-1} min^{-1}	$k_2 \times 10^{-3}$, l. mol^{-1} min^{-1}
Imidazole		7.6			6.4[b]
Imidazole acetic acid		7.7			2.9[c]
N-Acetyl-L-histidine		7.6			3.1[b]
L-Histidine	6.6	7.6	9.6	14.4[b]	2.8[b]
Histamine	6.4	7.5	10.0	24[c]	4.2[c]
Glycyl-L-histidine	7.2	7.6	10.0	5.0[c]	3.1[c]
β-Alanyl-L-histidine	7.4	7.6	10.0	4.6[b]	3.7[b]

[a] K_1, K_2, and K_3 are defined by the equations $N^+D_3Im^+DCOO^- \rightleftharpoons N^+D_3ImCOO^- + D^+$ (K_1), $ND_2Im^+DCOO^- \rightleftharpoons ND_2ImCOO^- + D^+$ (K_2), and $N^+D_3ImCOO^- \rightleftharpoons ND_2ImCOO^- + D^+$ (K_3) where the structures are defined in the text. A detailed discussion of the origin of these values is given elsewhere.[10] [b] At 37°. [c] At 35°.

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(2) H. S. Staab, *Tetrahedron Lett.*, 845 (1964).
(3) R. A. Olofson, W. R. Thompson, and J. S. Michelman, *J. Amer. Chem. Soc.*, **86,** 1865 (1964).
(4) T. M. Harris and J. C. Randall, *Chem. Ind. (London)*, 1728 (1965).
(5) J. D. Vaughan, Z. Mughrabi, and E. C. Wu, *J. Org. Chem.*, **35,** 1141 (1970).
(6) J. H. Bradbury and H. A. Scheraga, *J. Amer. Chem. Soc.*, **88,** 4240 (1966).
(7) J. H. Bradbury and P. Wilairat, *Biochem. Biophys. Res. Commun.*, **29,** 84 (1967).
(8) N. L. R. King and J. H. Bradbury, *Nature (London)*, **229,** 404 (1971).
(9) J. H. Bradbury and B. E. Chapman, *Biochem. Biophys. Res. Commun.*, **49,** 891 (1972).

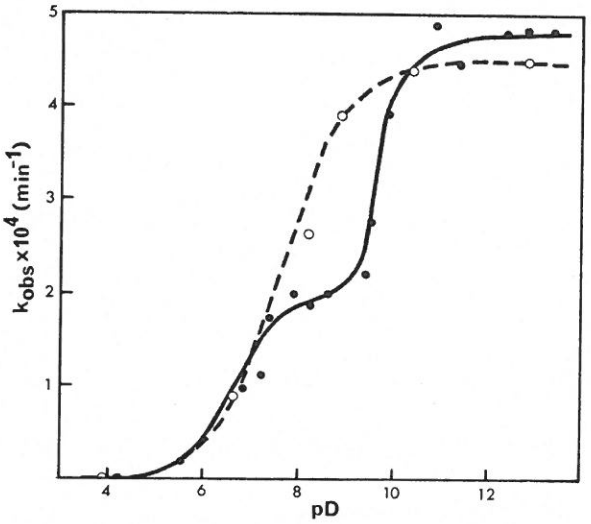

Figure 1. Graph of k_{obsd} (min^{-1}) *vs.* pD for *N*-acetyl-L-histidine (○) and L-histidine (●) at 37°.

rate of H–D exchange of 3–5% solutions of the compounds at various pD values (pD = pH meter reading + 0.4) was determined by following the decrease of the area (or height) of the C-2 proton resonances at 60 MHz as compared with the area (or height) of the C-4 proton resonances which remained constant.[5] A first-order rate constant k_{obsd} was determined from the gradient of a graph of log (corrected area or height of C-2 resonance) *vs.* time.[5,9] At pD <5 and 35° the rate of exchange is negligibly small; hence the reaction involving D_2O, which is appreciable at 65°,[5] can be neglected. Thus, for imidazole

$$\text{rate} = k_{obsd}[Im_t] = k_2[OD^-][Im^+] \quad (1)$$

where $[OD^-]$ is a constant in any particular run and $[Im_t]$ and $[Im^+]$ represent the total concentrations of imidazole and the charged form of imidazole, respectively. Substitution of the apparent dissociation constant of imidazole (K_2) and K_{D_2O}, the ionic product of D_2O, and rearrangement give

$$k_{obsd} = k_2K_{D_2O}/(K_2 + [D^+]) \quad (2)$$

This allows the determination of k_2 from measurements of k_{obsd} at different values of $[D^+]$.

For compounds which contain a separate nearby ionizable group with a pK of 6–12, the kinetics are complicated (see Figure 1) because of the different rate constants for the reaction of OD^- with the two forms of the compound. For example with histidine the two reactive forms are designated $N^+D_3Im^+DCOO^-$ (His^{2+}) and $ND_2Im^+DCOO^-$ (His^+), where the former structure represents the positively charged forms of the amino group and imidazole ring of histidine and the charged form of the carboxyl group. Thus

$$\text{rate} = k_{obsd}[His_t] = [OD^-](k_1[His^{2+}] + k_2[His^+]) \quad (3)$$

where $[His_t]$, $[His^{2+}]$, and $[His^+]$ represent the total concentrations of histidine and of the two reactive forms and k_1 and k_2 are second-order rate constants for the reactions of OD^- with His^{2+} and His^+, respectively. Substitution for $[His^{2+}]$ and $[His^+]$ in eq 3 in terms of K_1, K_2, and K_3 (defined in Table I) gives[10]

$$k_{obsd} = \frac{k_1K_{D_2O}}{K_1 + [D^+] + \frac{K_1K_3}{[D^+]} + \frac{K_1K_3}{K_2}} + \frac{k_2K_{D_2O}}{K_2 + [D^+] + \frac{K_2[D^+]}{K_3} + \frac{K_2[D^+]^2}{K_1K_3}} \quad (4)$$

By substitution of values for K_{D_2O}, K_1, K_2, K_3, and k_{obsd} at various values of $[D^+]$ a series of equations is obtained each with two unknowns, k_1 and k_2. Pairs of these equations are solved for k_1 and k_2 and the results (accuracy 5–10%) are given in Table I.

The S-shaped curve for *N*-acetyl-L-histidine shown in Figure 1 has been obtained hitherto[5] and the apparent pK of the imidazole can be determined from the center of the curve.[11,12] However, where there is a charged group nearby to the imidazole ring which titrates at pD >8, it is possible to obtain the pK of this group too, from the center of the second S-shaped curve, as shown for histidine in Figure 1.[13] This is useful for proteins such as ribonuclease A, in which there are charged amino groups nearby to histidines 12 and 119. Of greater importance for protein studies are conclusions obtained from examination of second-order rate constants. Firstly, the rate constant decreases greatly from the value of 14.4 in L-histidine, by moving the charged amino group progressively further away to a value of 5.0 in glycyl-L-histidine, 4.6 in β-alanyl-L-histidine, and finally 2.8 by removing the charge altogether as in L-histidine at high pD. Secondly, the rate constant increases greatly by eliminating a nearby charged carboxyl group as shown by comparing imidazole acetic acid with imidazole or L-histidine with histamine. Both effects are explained by a simple electrostatic mechanism in which the rate of attack of OD^- is increased by nearby positively charged groups and decreased by nearby negatively charged groups.

This study allows the determination of the pK of titratable groups (with pD >8) adjacent to imidazole rings and provides information on the proximity of nearby charged amino and carboxyl groups. The mapping of the environment of the histidine residues in ribonuclease A is in progress.

(10) J. H. Bradbury, B. E. Chapman, and F. A. Pellegrino, manuscript in preparation.

(11) B. E. Chapman, Ph.D. Thesis, Australian National University, 1972.

(12) H. Matsuo, M. Ohe, F. Sakiyama, and K. Narita, *J. Biochem. (Tokyo)*, 72, 1057 (1972).

(13) There is a further increase of k_{obsd} above pD 9, in spite of the much lower value of k_2 than k_1, because K_2 is much smaller than K_1; see Table I and eq 4.

J. H. Bradbury,* B. E. Chapman, F. A. Pellegrino
Chemistry Department, Australian National University
Canberra, A.C.T., Australia
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