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Quantitative Measurement of Solvent Accessibility of Histidine Imidazole Groups in Proteins

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Abstract

We report a method to express the solvent accessibility of histidine imidazole groups in proteins. The method is based on measuring the rate of hydrogen exchange (HX) reaction of the imidazole $C^{\varepsilon1}$-hydrogen. The rate profile of the HX reaction as a function of pH gives a sigmoidal curve, which reaches the maximum rate constant (k^{max}) on the alkaline side of the sigmoidal curve. To quantitatively describe the solvent accessibility of imidazole groups in proteins, it is necessary to compare the k^{max} of the imidazole groups with their intrinsic k^{max} (${}^{i}k^{max}$), the maximum rate constants for the given imidazole groups when they are fully exposed to the bulk solvent. However, the mechanism of HX reaction suggests that the ${}^{i}k^{max}$ of an imidazole group differs depending on its pK_a, and no systematic study has been conducted to clarify how the ${}^{i}k^{max}$ is affected by pK_a. We therefore investigated the relationship between ${}^{i}k^{max}$ and pK_a using four imidazole derivatives at three different temperatures. The experimentally determined pK_a-specific ${}^{i}k^{max}$ values allowed us to derive a general formula to estimate the ${}^{i}k^{max}$ value of any given imidazole group exhibiting a specific pK_a at a specific temperature. Using the formula, the protection factors (PF), the ratio of ${}^{i}k^{max}$ to k^{max}, of five imidazole groups in dihydrofolate reductase were obtained and used to express the magnitude of their solvent accessibility. In this definition, the smaller the PF value, the higher the solvent accessibility, and a value of 1 indicates full exposure to the bulk solvent. The solvent accessibility expressed by the PF values agreed well with the solvent accessible surface areas (ASA) obtained from the X-ray diffraction data.

INTRODUCTION

It was found more than four decades ago that the imidazole $C^{\varepsilon1}$-proton can be exchanged with deuteron by incubation in deuterium oxide (D_2O) (1). The Scheme 1 shows the reaction mechanism of the hydrogen exchange (HX) reaction. The HX reaction has been shown to follow pseudo-first-order kinetics, in which the abstraction of the $C^{\varepsilon1}$-proton from the

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SUPPORTING INFORMATION AVAILABLE
Temperature effect on the pH* of D_2O buffers, and structures of histamine, Ac-His-NHMe, Ac-His-OH, and IPA. This material is available free of charge via the Internet at http://pubs.acs.org.

cationic imidazolium by OD^- to form a ylide or a carbene intermediate is the rate-determining step (2–4). The rate profile of the HX reaction as a function of pD yields a sigmoidal curve, which exhibits a progression from the acidic side that accelerates and approaches the plateau on the alkaline side of the sigmoidal curve (Figure 1) (2–6). The rate profile provides two useful parameters that indicate the local environment of the given imidazole group in a protein. The one is the pK_a of the imidazole N-H group, which coincides with the inflection point of the sigmoidal curve, because the rate of the reaction depends on both the concentrations of the conjugate acid of imidazole and OD^- as can be seen below in the rate equation for this reaction (Equation 1). The other useful parameter is the maximum pseudo first-order rate constant, k^{max}, which corresponds to the upper plateau of the sigmoidal curve.

Because of the importance of imidazole groups in proteins such as enzyme catalysis and pH-dependent structural changes, there has been considerable interest in HX of imidazole groups in proteins (7). Until recently, proton nuclear magnetic resonance (^1H NMR) spectroscopy was the technique of choice to monitor HX reactions of imidazole groups in proteins (8). However, mass spectrometry (MS) is gaining popularity in recent years, because MS provides higher sensitivity and straightforward signal assignment, and is capable of analyzing membrane proteins and complex protein mixtures (7, 9–12). The MS method has been referred to as histidine hydrogen-deuterium exchange mass spectrometry (His-HDX-MS) (9–11) in order to distinguish it from the amide-HDX-MS (13).

Because the pK_a value of an imidazole group is sensitive to neighboring charged groups, experimentally obtained pK_a values are useful indicators of the electrostatic environment of imidazole groups in the proteins (11). On the other hand, k^{max} values have been used to indicate the solvent accessibilities of imidazole groups (7, 9, 11, 14, 15), because the HX rates at individual imidazole groups are affected significantly by the extent of accessibilities of these groups to the bulk solvent. However, the relationship between the HX rate and solvent accessibility is not straightforward. According to the kinetic studies of the HX reaction (2–4, 6), the reaction rate is expressed by Equation 1:

$$\text{rate}=k[\text{Im}_{\text{total}}]=k_2[\text{Im}^+][\text{OD}^-] \qquad 1)$$

where k is the pseudo first-order rate constant; k_2 is the second order rate constant for the rate-determining step of the HX reaction; $[\text{Im}_{\text{total}}]$ and $[\text{Im}^+]$ represent the concentrations of total imidazole and the charged form of imidazole, respectively; and $[\text{OD}^-]$ is the concentration of deuteroxyl ions. Because

$$[\text{Im}^+]=\frac{[\text{Im}_{\text{total}}][\text{D}^+]}{(K_a+[\text{D}^+])}$$

the expression of k can be simplified to Equation 2.

$$k=\frac{k_2 k_w}{K_a+[\text{D}^+]} \qquad 2)$$

where K_a is the dissociation constant of the imidazolium cation and K_W is the ion product of heavy water ($K_W = [\text{D}^+][\text{OD}^-] = 10^{-14.95}$ at 25°C) (16). The equation indicates that the magnitude of the k value is influenced by the K_a of a given imidazole group and the pD at which the HX reaction takes place. At high pD, however, $[\text{D}^+]$ is negligibly smaller than the K_a; therefore, Equation 2 reduces to Equation 3.

$$k^{\max}=\frac{k_2 K_w}{K_a} \quad 3)$$

where $k^{\max}$ is the maximum k obtained from the upper plateau of the sigmoidal curve in Figure 1. As Equation 3 implies, the $k^{\max}$ is independent of pD, and is therefore a more straightforward indicator of solvent accessibility than is k. The value of $k^{\max}$ is proportional to k_2 and inversely proportional to K_a; therefore, it increases 10-fold with a pK_a increase of one pH unit, if the k_2 value remains the same (which is not actually the case). This means that we cannot simply assume that a high value of $k^{\max}$ is due to greater accessibility of an imidazole group in the protein, because it may be simply due to a high value of pK_a. Therefore, to quantitatively express the solvent accessibility of a given imidazole group in a protein, it is necessary to know its intrinsic $k^{\max}$ (${}^{i}k^{\max}$) value, which is the $k^{\max}$ value when it is fully exposed to the bulk solvent, and the value is specific to its pK_a value. Such an ${}^{i}k^{\max}$ – pK_a relationship for the HX method has not been investigated in a systematic manner, although some studies for the closely related hydrogen-tritium exchange (HTX) method have been done (17).

We report here the relationship between ${}^{i}k^{\max}$ and pK_a obtained with four imidazole derivatives. Based on the study, we derived a general formula that allows us to estimate the ${}^{i}k^{\max}$ value of an imidazole group in a protein exhibiting a specific pK_a. The formula was used to obtain the protection factors (PF), which is defined as the ratio of ${}^{i}k^{\max}$ to $k^{\max}$ of a given imidazole group, to express the solvent accessibility of imidazole groups in a protein.

EXPERIMENTAL PROCEDURES

Materials

Deuterium oxide (D_2O, 99%) was purchased from Cambridge Isotope laboratories (Andover, MA). Histamine, N^{α}-acetyl-DL-histidine (Ac-His-OH), and 1H-imidazole-5-propanoic acid (IPA) were from Sigma-Aldrich (St. Louis, MO). N^{α}-acetyl-L-histidine methylamide (Ac-His-NHMe) was from Bachem (Torrance, CA). All other chemicals and materials used were either reagent grade or of the highest quality commercially available.

Buffer Solutions in D_2O

Buffers in D_2O contained 100 mM pyridine (pH* 4.82–6.51) and 100 mM N-ethylmorpholine (pH* 6.62–9.39). The pH* of the buffer was adjusted with diluted monodeuteroacetic acid (CH_3COOD, 99%) at 25°C with a Solution Analyzer model 4603 (Amber Science, Eugene, OR) equipped with a glass AgCl electrode (model 476086, Nova Analytics, Woburn, MA). The reported pH* values are direct pH meter readings of the D_2O buffer solutions calibrated with standard buffer solutions made with H_2O and are uncorrected for the isotope effect at the glass electrode. The pH* of the D_2O buffers at different temperatures were estimated as described in the Supporting Information.

Deuteration of Imidazole Derivatives

One nanomole of histamine, Ac-His-NHMe, Ac-His-OH, or IPA was incubated nμL buffer in D_2O with different pH* values (4.82–9.39) at 25, 31, and 37°C for 192, 106, and 50 hr, respectively (see the Supporting Information for the structures of these four model compound). The reaction was stopped by the addition of 5 μL formic acid, and the samples were dried in a Speed Vac. Note that the reaction is negligibly slow at the acidic pH* (see Figure 1). Prior to MS analysis, the dried samples were redissolved in 50 μL of 0.1% formic acid/50% methanol in H_2O, which promotes deuterium to proton exchange at rapidly exchangeable sites (the amino, amide NH and carboxyl groups). The samples are then subjected to flow injection MS analysis as described below.

Mass Spectrometry

The samples prepared above were analyzed using a QStar Elite quadrupole/time-of-flight mass spectrometer equipped with a TurboIonSpray® ion source (AB Sciex, Framingham, MA, USA). A 10-μL aliquot of each sample was injected into a flowing carrier stream consisting of 0.1% formic acid/50% methanol at 40 μL/min, which was directly introduced into the ion source of the mass spectrometer. A Shimadzu LC-20AD pump (Columbia, MD, U.S.A.) was used to deliver the carrier solvent. Mass spectra (from m/z 100 to 300) were acquired in the time-of-flight mass analyzer with 1 sec accumulation time. Analyst QS software (version 2.0, AB Sciex) was used for instrument control, data acquisition, and data processing.

Measurement of HX Rate Constant (k) and pK_a

The pseudo-first-order rate constant (k) of the HX reaction was determined by monitoring changes in the ratios of the M+1/M isotopic peak of a given imidazole derivative before (time = 0) and after (time = t) the HX reaction as described previously (11). The pK_a values of imidazole derivatives were obtained from the sigmoid titration curve of k versus pH* using Origin Graphing software (version 8.5, OriginLab, Northampton, MA) using the following equation.

$$y=A_2+\frac{A_1-A_2}{1+e^{(x-x_0)/\delta_x}}$$

where A_1 is the minimum rate constant at the lowest pH*, A_2 is the maximum rate constant at the highest pH*, x_0 is the point of inflection, and δ_x is the change in x corresponding to the most significant change in y values.

RESULTS AND DISCUSSION

Relationship between $^{i}k^{max}$ and pK_a

Figure 2a shows the rate profiles of the HX reactions of histamine, Ac-His-NHMe, Ac-His-OH, and IPA at 25°C as a function of pH*. The pK_a values of these four model compounds obtained from the inflection point of the sigmoidal curves were: 5.42 for histamine, 6.35 for Ac-His-NHMe, 7.38 for Ac-His-OH, and 7.77 for IPA. This suggests that a neighboring amino group lowers the pK_a of imidazole group as in the case of histamine, whereas a neighboring carboxyl group raises the pK_a as in the case of Ac-His-OH and IPA. This result is consistent with the well-established fact that neighboring electron-withdrawing groups (e.g., RNH_3^+, $GdnH^+$, ImH^+) lower the pK_a of the imidazole group, whereas neighboring electron-donating groups (e.g., $RCOO^-$, RO^-, RS^-) raise it (18). The pK_a of Ac-His-NHMe is considered to be the intrinsic pK_a of the imidazole group in proteins that have no adjacent dissociable groups.

The rate profiles in Figure 2a also provide the maximum k values (k^{max}) for the imidazole derivatives. The obtained values were 0.0016, 0.0029, 0.0057, and 0.0104 (hr^{-1}) for histamine, Ac-His-NHMe, Ac-His-OH, and IPA, respectively. As expected from the reaction mechanism (2–4, 6), the k^{max} increased with increasing pK_a in these four model compounds. Because the imidazole groups of these compounds are fully exposed to the bulk solvent, these k^{max} values can be considered to be their intrinsic k^{max} ($^{i}k^{max}$) values. In the base-catalyzed reaction, the plot of the common logarithm of the reaction rate constant, log*k*, versus the pK_a, gives a straight line with slope β and intercept logC, which is known as the Brønsted relation. A plot of the log$^i k^{max}$ values of these four model compounds against their pK_a values at 25°C yielded a straight line with a slope of 0.3255 and an intercept value of −4.5797, as shown in Figure 2b. It is, therefore, confirmed that the HX reaction of imidazole $C^{\epsilon 1}$-hydrogen fulfills the Brønsted catalysis equation.

General formula to calculate the pK$_a$-specific ^{i}k$_{max}$ values of imidazole groups at a specific temperature

Although the equation obtained above can be used to find the pK_a-specific log$^i k^{max}$ values of imidazole groups at 25°C, the HX reaction may be carried out at a different temperature. To obtain a general formula to calculate the pK_a-specific log$^i k^{max}$ values of imidazole groups at any specific temperature, we determined the $^i k^{max}$ values for the four model compounds at 31 and 37 °C as well (Table 1). The $^i k^{max}$ values of the model compounds at each temperature were converted to log$^i k^{max}$ and plotted against their pK_a values (Figure 3a). The magnitude of the log$^i k^{max}$ values increased as the temperature increased; however, as can be seen, the slopes of the three linear regression lines remained unchanged. We therefore averaged the three slope values, yielding a value of 0.3159, and used this in the general formula given below. In addition to the common slope value, the general formula requires the temperature-specific y-intercept values. The relationship between the y-intercept and temperature was obtained by plotting the y-intercept values at pK_a = 7.0 from the three linear regression lines against the temperature, as shown in Figure 3b. Note that the y-intercept values at pK_a = 7.0 were used, because they are much closer to the pK_a values of typical imidazole groups in proteins than those at pK_a = 0, and are thus expected to provide more accurate log$^i k^{max}$ values around typical pK_a values of imidazole groups. From the plot, the relationship between the y-intercept at pK_a = 7.0 and temperature (T) was found to be:

$$y=0.0576 \times T-3.7467$$

where y is the y-intercept value at pK_a = 7.0 and T is the temperature in Celsius. Thus, the general formula to calculate the pK_a specific log$^i k^{max}$ value of an imidazole group at a specific temperature was derived to be:

$$log^i k^{max}=0.3159 \times (pK_a-7)+(0.0576 \times T-3.7467)$$

and, inverting the logarithm function gives us $^i k^{max}$ value.

Quantitative analysis of solvent accessibilities of five histidine imidazole groups in DHFR

We recently reported the pK_a and k^{max} of five histidine imidazole groups in dihydrofolate reductase (DHFR) (11). The data was obtained at 37°C. The experimentally determined pK_a and k^{max} values of the five imidazole groups in apo-DHFR, and their $^i k^{max}$ values at 37°C calculated using the general formula are shown in Table 2. On the basis of solely the k^{max} values, the most reactive residue is His141 and the reactivity decreases in the following order: His141 > His45 > His149 > His 124 > His114. However, we cannot simply assume that the decreasing order of reactivity is same as the decreasing order of solvent accessibility, because the magnitude of their intrinsic reactivities (rate constants), $^i k^{max}$, are influenced significantly by their pK_a values. Therefore, in order to accurately describe the solvent accessibilities of the histidine imidazole groups, we propose to use the protection factors (PF) defined as the ratio of the HX rate of a given histidine imidazole group when it is in a fully solvent-exposed state ($^i k^{max}$) to the HX rate in the protein (k^{max}) as shown below.

$$PF=\frac{{}^{i}k^{max}}{k^{max}}$$

In this definition, the smaller the value, the higher the solvent accessibility, and a value of 1 indicates full exposure to the bulk solvent. Now it is evident in the table that the true solvent accessibility decreases in the following order: His45 ≈ His149 > His141 > His 124 > His114, which is different from the order obtained based solely on the reactivity of these residues ($\log k^{\max}$ values) described above. Analogous PF concepts are used in amide-HX, especially in NMR studies (19).

The straight line in Figure 4 shows the relationship between $\log {}^{i}k^{\max}$ and pK_a ($\log {}^{i}k^{\max} - pK_a$ plot) at 37°C produced using the general formula. In the figure, the $\log k^{\max}$ values of the five imidazole groups in DHFR are plotted. The solvent accessibilities of the five imidazole groups are indicated by the distance from the straight line of the plot to the plotted individual $k^{\max}$ values. On the plot, the shorter the distance, the higher the solvent accessibility, and the presence of any plotted point on the straight line indicates that the corresponding imidazole group is fully exposed to the bulk solvent. For easy understanding, the PF values for these histidine residues from Table 2 are also shown. We found that this type of plot with the PF values is convenient to visually indicate the solvent accessibility of imidazole groups in proteins.

Figure 5 shows how the solvent accessibilities of five imidazole groups in apo-DHFR (black circles) change upon folate and $NADP^+$ binding. The experimentally determined pK_a and $k^{\max}$ values of the five imidazole groups in DHFR-folate-$NADP^+$ are shown in Table 2. It is clearly seen in the plot that the solvent accessibilities of His114 and His124 decrease upon ligand binding, whereas the solvent accessibility of His45 increases, accompanied by an increased pK_a. Thus, the $\log {}^{i}k^{\max} - pK_a$ plot is useful to visualize the changes of solvent accessibility and pK_a of imidazole groups in proteins.

It is worth mentioning that one can carry out an experiment measuring the ${}^{i}k^{\max}$ values of imidazole groups in a completely unfolded protein, and compares the values with the $k^{\max}$ values in the folded protein to obtain the PF values. Such approaches are effective in estimating the intrinsic HX rates for backbone amide N-H groups (20), which are significantly influenced by the two adjacent side chains even in the absence of folded structure (21). This is because the two adjacent side chains have different degrees of steric hindrance and inductive effects to the amide N-H group between them. However, in case of His-HX the values obtained by such experiments do not accurately reflect the ${}^{i}k^{\max}$ values of these imidazole groups because of the following two reasons. First, the pK_a of imidazole groups in the unfolded protein are different from those in the folded protein due to the loss of electrostatic effect by the neighboring side chains and other structural elements in the three dimensional geometry, which exists only in the folded state. Second, in contrary to the amide HX, no steric hindrance by the adjacent side chains to the imidazole groups in unfolded structure is expected, though they may elicit inductive effect. Such inductive effect, however, should alter the pK_a of the imidazole ring, and therefore is taken into account in the pK_a specific ${}^{i}k^{\max}$ values. Thus, the ${}^{i}k^{\max}$ values of imidazole groups need to be determined by the model compounds with different pK_a values whose imidazole groups are fully solvent exposed.

Correlation of the PF values with solvent accessible surface areas obtained from X-ray diffraction data

The solvent accessible surface areas of the $C^{\varepsilon 1}$ atom of five histidine imidazole groups in apo-DHFR and DHFR-folate-NADP$^+$ complex are shown in Table 2. Note that the smaller the PF values, the higher the solvent accessibility; while the larger the ASA values, the higher the solvent accessibility. On the basis of the PF values for the histidine residues in apo-DHFR, the solvent accessibility decreases in the following order: His45 ≈ His149 > His141 > His 124 > His114, while ASA data suggests the following order: His45 > His141 > His 124 > His114 ≈ His149. The only difference is His149, for which the PF value indicates that the residues is quite accessible to the bulk solvent, yet the ASA data indicates that His149 is the least solvent accessible residue. This discrepancy may be reflecting the difference in protein structures in the solution and crystal. We performed a two-sample Wilcoxon rank-sum test (22) for assessing whether the solvent accessibility ranked by the PF and ASA are statistically different. The analysis for the two rankings gave the Pearson's Correlation Coefficient of 0.53 with the p-value for the Wilcoxon rank-sum test of 0.84 (the null hypothesis was that the distributions of PF and ASA are the same), indicating that the difference between the two rankings is not significantly different. Likewise, the solvent accessibility ranking of these histidine residues by the PF and ASA in DHFR-folate-NADP$^+$ complex appeared quite similar to each other as can be seen in Table 2 in the following order: His45 > His141 ≈ His149 > His124 > His114. The statistical analysis for the two rankings gave the Pearson's Correlation Coefficient of 0.93 with the p-value for the Wilcoxon rank-sum test of 0.15. Thus, the solvent accessibilities indicated by the PF values appear to agree well with ASA data. It should be noted, however that the ASA values we used were for only the $C^{\varepsilon 1}$ atom of imidazole group. When we used the ASA values for entire imidazole ring we did not find good correlation with the FA values. The finding suggests that it is not necessary for an imidazole group to be entirely exposed to the bulk water for the HX to occur. Further accumulation of His-HX data will clarify this point.

Even though the data size is small, the decent correlation between the PF and SAS value suggests that the PF value reflects the solvent accessibility of imidazole groups in proteins. This is contrary to amide HX, in which the critical event that allows exchange is hydrogen-bond separation and exposure, not solvent access to the amide N-H group (23). More data on PF will be needed to confirm the positive correlation between the PF and SAS.

Temperature dependence of the $^{i}k^{max}$

The $^{i}k^{max}$ values for the four model compounds at different temperatures presented in Table 1 were used to generate Arrhenius plots (Figure 6). From the plots the activation energies of the HX reaction for histamine, Ac-His-NHMe, Ac-His-OH, and IPA were estimated to be 104, 106, 102, and 99 kJ/mol, respectively. These values agree well with the value of 104 kJ/mol obtained with L-histidine at pH* 9 (6), at which the obtained rate constant can be considered to be $^{i}k^{max}$.

In summary, we have described a method to measure the solvent accessibility of histidine imidazole groups in proteins. The key assumption underlying the analysis is that the structure and solvent accessibility does not change over the pH range investigated. It can easily be tested whether the assumption holds by comparing the experimentally obtained titration curves with the theoretical curves. For example, the titration curves of the four model compounds can be superimposed perfectly on their theoretical curves, suggesting no cooperative conformational rearrangement occurred on these compounds over the pH range investigated. However, it may not be unusual to find imidazole groups in proteins whose titration curves are not superimposed on the theoretical ones. Such non superimposable titration curves are indicative of the pH dependent changes of local electrostatic or hydrophobic environment, or both, of the given imidazole group. Apparently, the PF values cannot be given to the buried non titratable imidazole groups. However, based on the analytical limit of the measurement, the data can provide information on "how slow the HX rates are" by providing the half-lives ($t_{1/2}$) of their HX reactions (9, 11). The measure of solvent accessibility expressed as PF value is an independent value and can be compared with the values of other imidazole groups in the same protein or in other proteins. The present method is intended to be used for the data obtained by mass spectrometry, however could also be used for the HX data obtained by ^{1}H NMR spectroscopy. The $\log{}^{i}k^{max} - pK_a$ plot is useful to show visually the solvent accessibilities of imidazole groups in proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Ac-His-OH	N^{α}-acetyl-DL-histidine
Ac-His-NHMe	N^{α}-acetyl-L-histidine methylamide
ASA	solvent accessible surface areas
DHFR	dihydrofolate reductase
HX	hydrogen exchange
IPA	1H-imidazole-5-propanoic acid
MS	mass spectrometry
NADP$^+$	nicotinamide-adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
PF	protection factor

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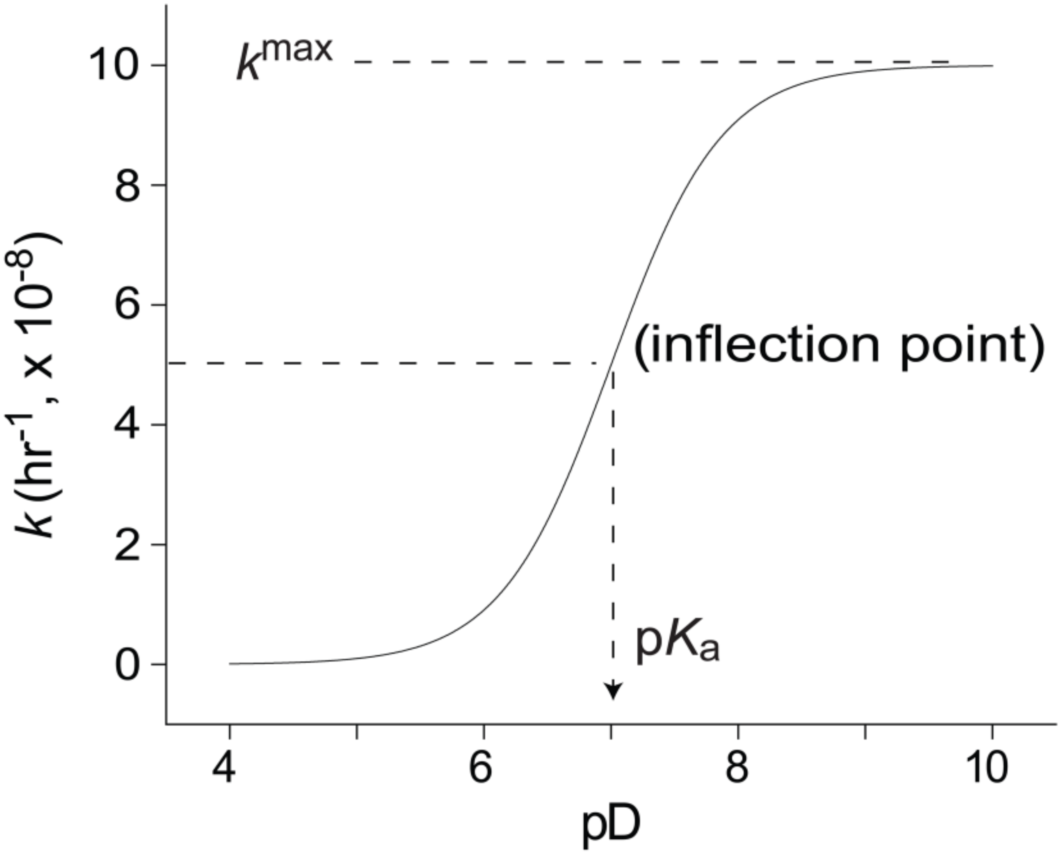


Figure 1.

A hypothetical rate profile of the HX reaction of the imidazole C$^{\varepsilon 1}$-hydrogen, plotting the pseudo first-order rate constant (k) against pD. The rate profile was produced by computing the k against varying pD using the equation, $k = k_2K_W/(K_a + [D^+])$, where k_2 is the second order rate constant for the rate-determining step of the HX reaction; K_W is the ion product of heavy water, and K_a is the dissociation constant of the imidazolium cation. The k_2, K_W and K_a were set to be 1 (mol^{-1}•hr^{-1}), 10–14 (mol^2/L) and 10^{-7} (mol/L), respectively, in this simulation. The pK_a of the imidazole group can be obtained from the inflection point of the sigmoidal curve, and the maximum pseudo first-order rate constant, k^{max}, from the upper plateau of the sigmoidal curve.

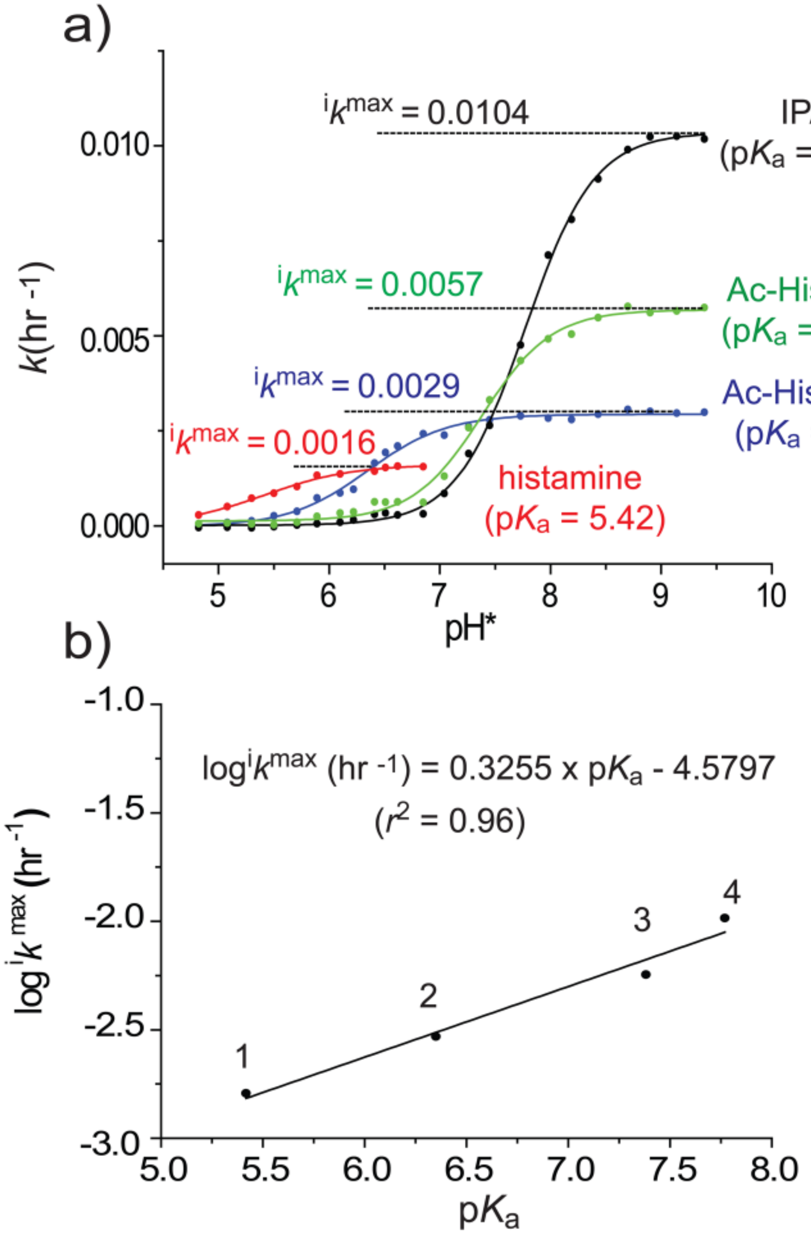


Figure 2.

pH* and pK_a dependence of HX reaction at the $C^{\epsilon 1}$-position of imidazole groups. (a) pH* dependence of the k for HX at the imidazole group in histamine (red), Ac-His-NHMe (blue), Ac-His-OH (green), and IPA (black). The experimentally obtained pK_a and the intrinsic k^{max} ($^{i}k^{max}$) for the four imidazole derivatives are shown. (b) log$^{i}k^{max}$ – pK_a plot for the HX reaction at the $C^{\epsilon 1}$-position of imidazole at 25°C in 1, histamine; 2, Ac-His-NHMe; 3, Ac-His-OH; and 4, IPA. The equation and the correlation coefficient (r^2) of the linear regression line are presented.

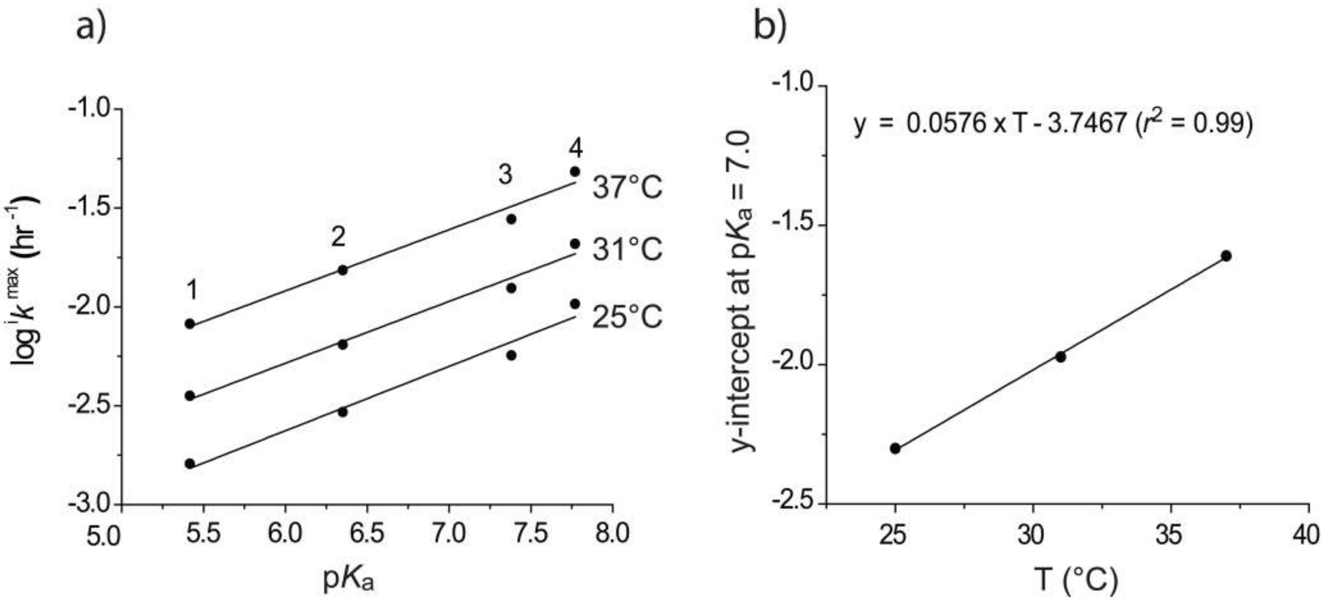


Figure 3.

$\log {}^{i}k^{max} - pK_a$ plots at different temperatures for the HX reaction at the $C^{\varepsilon 1}$-position of imidazole. (a) 1, histamine; 2, Ac-His-NHMe; 3, Ac-His-OH; and 4, IPA. The linear regression analyses for 25, 31, and 37°C plots yielded slope values of 0.3255, 0.3129, and 0.3094, and y-intercept values (at $pK_a = 7.0$) of −2.3001, −1.9723, –and −1.6097, respectively. (b) The relationship between the y-intercept value and temperature is shown. The y-intercept values at $pK_a = 7.0$ were obtained from Figure 4a. The equation and the correlation coefficient (r^2) of the linear regression line are presented.

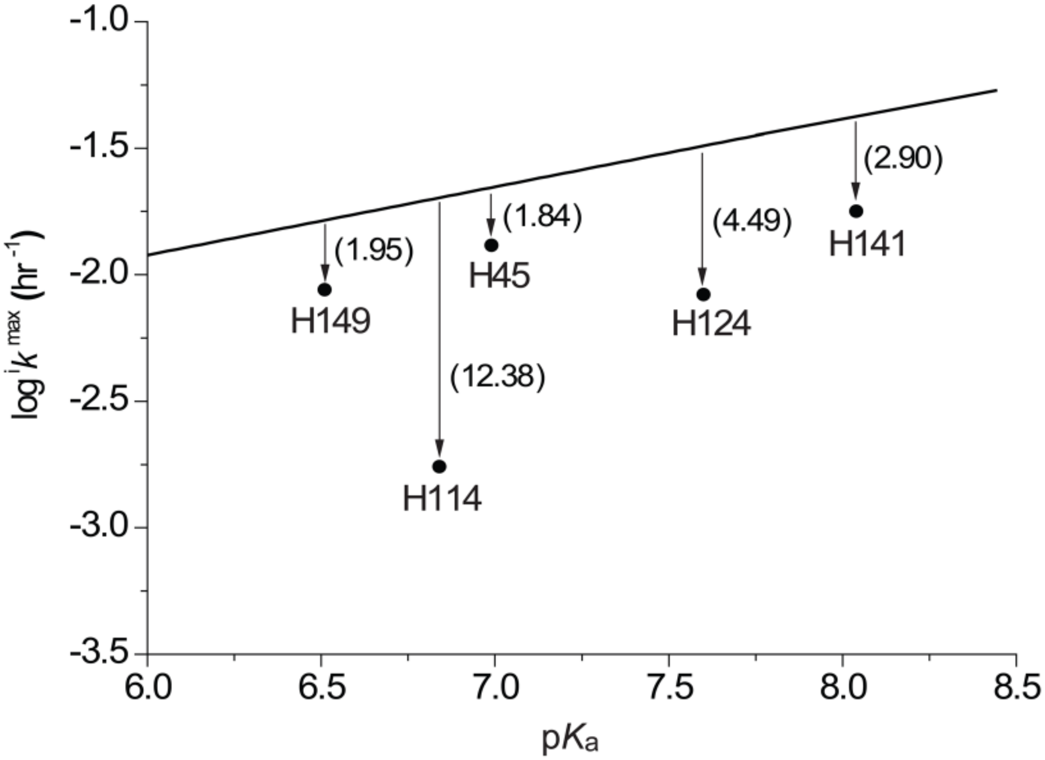


Figure 4.
$\log k^{\max}$ values of five histidine imidazole groups in apo-DHFR, plotted on the $\log {}^{i}k^{\max}$ – pK_a plot at 37°C. The straight line in the figure was produced using the formula: $\log {}^{i}k^{\max} = 0.3159 \times (pK_a - 7) + (0.0576 \times 37 - 3.7467)$. The black circles are $\log k^{\max}$ values of five histidine imidazole groups in apo-DHFR. The numbers in the parentheses are PF values for the imidazole groups.

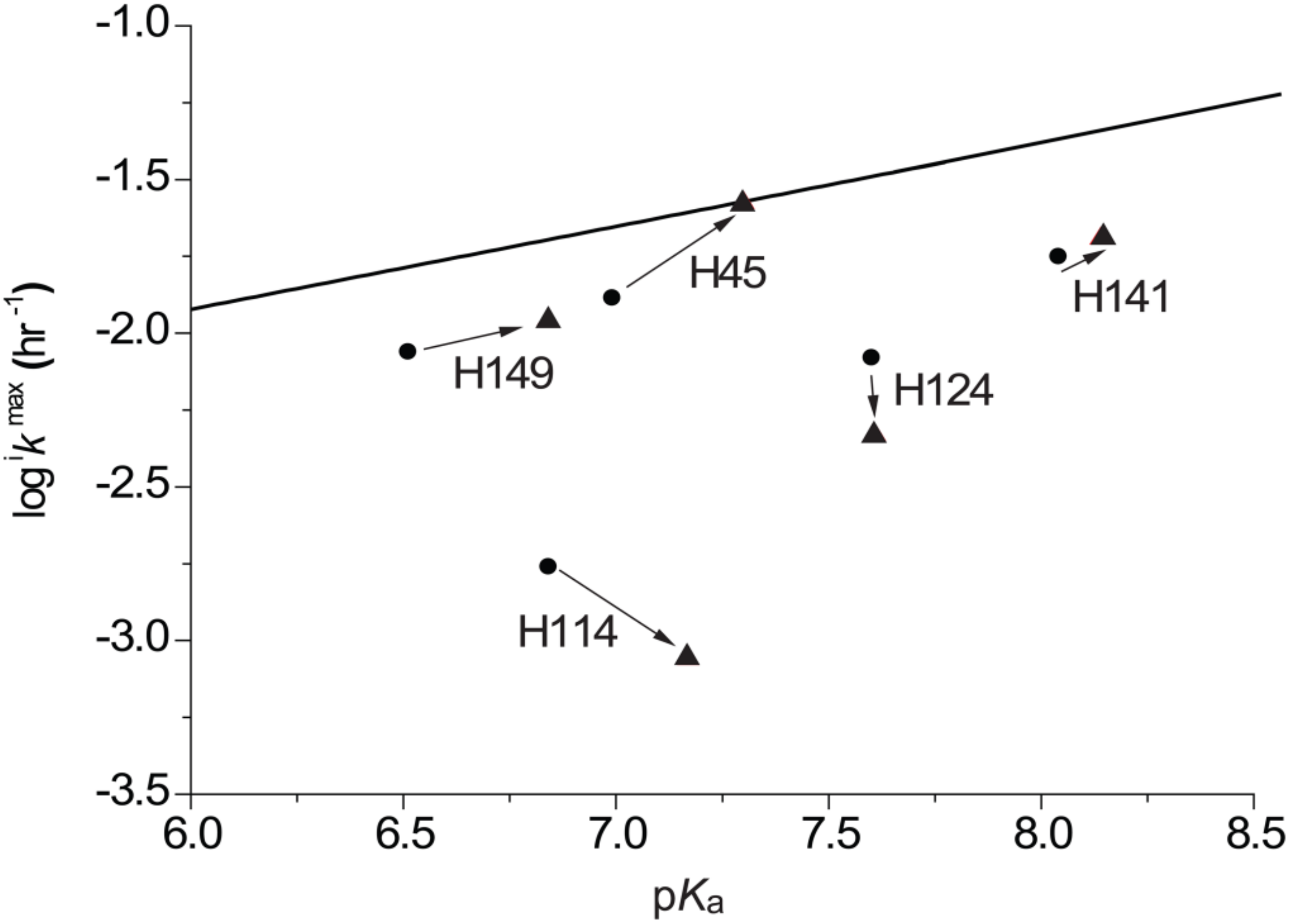


Figure 5.

Changes in solvent accessibilities of five histidine imidazole groups in apo-DHFR upon folate and NADP+ binding. The $\log k^{max}$ values of five histidine imidazole groups in apo-DHFR (circles) and DHFR-folate-NADP+ complex (triangles) are plotted on the $\log {}^{i}k^{max}$ – pK_a plot at 37°C. The straight line in the figure was produced using the formula: $\log {}^{i}k^{max} = 0.3159 \times (pK_a - 7) + (0.0576 \times 37 - 3.7467)$.

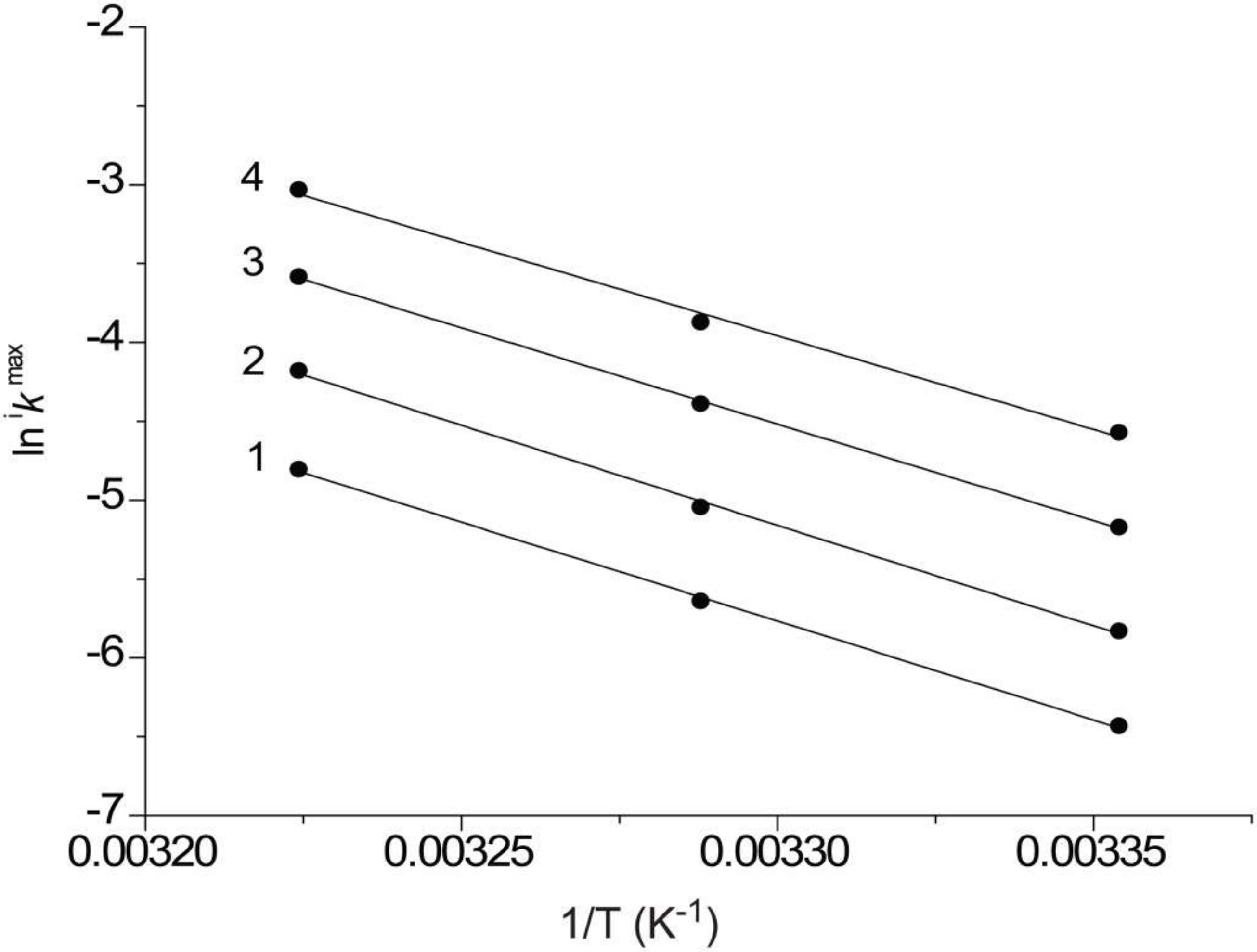
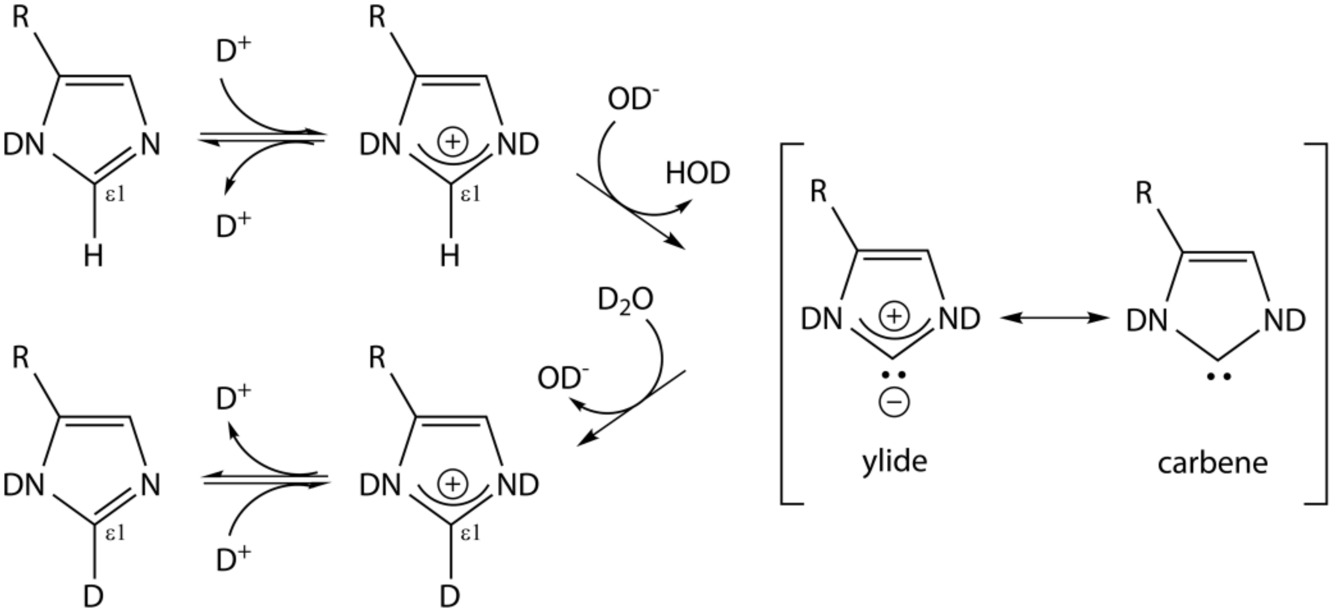


Figure 6.

Arrhenius plots of the HX reaction at the C$^{\varepsilon1}$-position of the imidazole groups of 1, histamine; 2, Ac-His-NHMe; 3, Ac-His-OH; and 4, IPA.



Scheme 1.

Mechanism of the HX reaction at the imidazole $C^{\varepsilon 1}$-hydrogen of histidine.

Table 1

$^{i}K^{max}$ values of imidazole derivatives at different temperatures

	[a] pK_a	$^{i}k^{max}$ (hr^{-1})		
		25°C	**31°C**	**37°C**
Histamine	5.42 (±0.11)	0.0016 (±0.0001)	0.0036 (±0.0002)	0.0082 (±0.0009)
Ac-His-NHMe	6.35 (±0.03)	0.0029 (±0.0001)	0.0065 (±0.0002)	0.0153 (±0.0003)
Ac-His-OH	7.38 (±0.02)	0.0057 (±0.0001)	0.0124 (±0.0003)	0.0278 (±0.0005)
IPA	7.77 (±0.01)	0.0104 (±0.0001)	0.0208 (±0.0003)	0.0483 (±0.0011)

[a] The pK_a values were values obtained at 25°C from the pH* dependence of the measured exchange rate.

Table 2

Solvent accessibilities of five imidazole groups in apo-DHFR and DHFR-folate-NADP$^+$

	pK_a	**k^{max} (hr^{-1})^a**	**$^ik^{max}$ (hr^{-1})^b**	**PF^c**	**ASA^d**
apo-DHFR					
His45	6.99	0.0131	0.0241	1.8	46.0
His114	6.84	0.0017	0.0216	12.4	16.4
His124	7.60	0.0083	0.0375	4.5	25.1
His141	8.04	0.0178	0.0516	2.9	38.7
His149	6.51	0.0087	0.0170	1.9	16.1
DHFR-folate-NADP$^+$					
His45	7.30	0.0265	0.0301	1.1	51.5
His114	7.17	0.0009	0.0274	31.5	17.0
His124	7.61	0.0047	0.0378	8.1	26.2
His141	8.14	0.0203	0.0555	2.7	35.7
His149	6.84	0.0111	0.0216	1.9	35.1

[a] Calculated from the reported $t_{1/2}$ (day) values using the equation: $K^{max} = \ln 2/t_{1/2}/24$.

[b] Calculated using the formula: $^ik^{max} = 10\text{^}\log{}^ik^{max}$.

[c] Calculated using the formula: PF = $^ik^{max}/k^{max}$.

[d] Solvent accessible surface area; the structural data of apo-DHFR (PDB code number: 5DFR) and DHFR-folate-NADP$^+$ (PDB code number: 1RX2) were used to obtain the ASA for the $C^{\varepsilon 1}$ atom of the five histidine residues.