NMR Site-to-Site Rate Constants and the Mechanisms of Acid-Catalyzed Proton Exchange in Secondary Amides

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Abstract: Kinetics of acid-catalyzed proton exchange have been determined for five secondary formamides. Since these exist as a mixture of *E* and *Z* stereoisomers, it is possible to distinguish the N-protonation mechanism from the imidic acid mechanism. Detailed mechanistic analysis centers on comparison of the rate constant k_{ZE} for stereoisomerization with the rate constants k_{ZS} and k_{ES} for intermolecular proton exchange, since only the N-protonation mechanism permits a nonzero k_{ZE}. The experimental method involves NMR measurement of site-to-site rate constants by a combination of saturation transfer and line-shape analysis. For simple N-alkylformamides it is concluded that exchange occurs by a mixture of the two mechanisms, but amides with electron-withdrawing substituents, such as *N*-carboxymethyl, *N*-phenyl, and α-cyano, exchange >99% via the imidic acid. In particular, it is concluded that NH protons of protein backbones exchange almost exclusively via the imidic acid, rather than by N-protonation as had long been assumed. The substituent effects and stereochemical preferences are discussed in terms of transition-state structures.

Introduction

Proton exchange in amides[1] has long been of wide interest, especially since proton-exchange kinetics of amides, peptides, and proteins can provide information about the structures of peptides and proteins in solution.[2] The mechanism of the acid-catalyzed exchange had long been accepted as involving N-protonation (eq 1), as originally suggested by Berger, Loewenstein, and Meiboom.[3]

$$\mathrm{RCONHR' + H^+ \rightleftharpoons RCONH_2{}^+R'} \qquad (1)$$

However, an alternative mechanism involving the imidic acid (eq 2) seems more reasonable, since it avoids protonating the amide

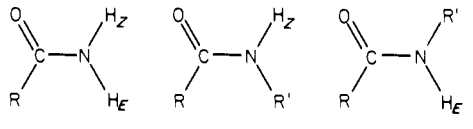
$$\mathrm{RCONHR' + H^+ \rightleftharpoons RC(OH){=}NHR'^+ \rightleftharpoons RC(OH){=}NR' + H^+} \qquad (2)$$

on nitrogen, which is ca. 10^7-fold less basic than the oxygen.[4] In view of long-standing interest in amide/imidic acid tautomerism,[5] this mechanism is worth pursuing.

Various attempts have been made to distinguish these mechanisms,[6] including our own. Initially, on the basis of a greater reactivity of H_E in primary amides (**1**), we were astonished to conclude[7] that exchange does occur via N-protonation, but with the additional feature that deprotonation of the intermediate $RCONH_3{}^+$ is competitive with rotation about its C–N single bond.[8] Subsequently we have used saturation-transfer techniques[9] to show that the occurrence of acid-catalyzed intramolecular exchange is strong evidence for the N-protonation mechanism in many primary amides. These techniques also show that intramolecular exchange in amides with electron-withdrawing substituents is significantly slower than intermolecular exchange, and we have interpreted that as the first unambiguous evidence for the imidic acid mechanism.[10]

Although primary amides are certainly of interest, peptides and proteins consist chiefly of secondary amides, whose mechanism(s) of proton exchange ought to be elucidated.[11] On the basis of substituent effects in *N*-methylacetamides,[12] we have concluded that, as with primary amides, the mechanism depends on substitution. Electron-donating substituents in R promote the N-protonation mechanism (eq 1), whereas electron-withdrawing substituents in R favor the imidic acid mechanism (eq 2). In particular, R = $CH_3CONHCH_2$ is classed as electron-withdrawing, so we concluded that the NH protons of proteins undergo acid-catalyzed exchange predominantly by the imidic acid mechanism. Yet such arguments based on substituent effects rely on analogy and cannot be definitive. Besides, the experimental results were limited to *N*-methylacetamides, and proteins have a different substituent on the nitrogen. We have therefore sought unambiguous evidence.

One absolute distinction between the two mechanisms is that only the N-protonation mechanism permits intramolecular proton exchange or *E*/*Z* isomerization. For primary amides (**1**) it can



1 2E 2Z

be shown[8b,10b] that intramolecular and intermolecular rate con-

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stants must satisfy $k_{ZE} = k_{ZS} < k_{ES}$, where k_{ij} is the rate constant for proton exchange from site i to site j ($i, j = E$, Solvent). For secondary amides, RCONHR′, the kinetics become more complicated, but an analogous, qualitative conclusion holds: secondary amides exist as a mixture of (*E*) and (*Z*) isomers (**2E** and **2Z**), which interconvert slowly enough that they show separate NMR spectra. (It should be noticed that consistency with primary amides requires that the (*E*) amide bear H_Z and vice versa. Also, the rate constant k_{ij} refers to exchange from proton site i to proton site j, rather than from isomer i to isomer j.) N-Protonation permits not only proton exchange but also E/Z interconversion, which thus becomes diagnostic for this mechanism. Unfortunately, the proportion of the (*E*) isomer is usually very low. For example, in *N*-methylacetamide there is only 3% of this isomer,[13] too little to permit adequate measurement of its rates of proton exchange and isomerization. Only by reducing the steric interaction between R and R′ is it possible to have an appreciable proportion of the (*E*) isomer. Thus this study is restricted to *N*-alkylformamides and similar compounds.

Comparison of proton exchange with E/Z isomerization requires measuring site-to-site rate constants. Depending on the amide, the site may be NH or CH of R′ or CH of R. Since NH peaks are ordinarily broadened by quadrupolar relaxation, the use of viscous solvents is sometimes required to relax the ^{14}N nucleus and sharpen the NH peaks. In simple cases where intermolecular proton exchange is much faster than E/Z isomerization, line-shape analysis of an adjacent CH doublet may suffice. In more complicated cases, the site-to-site rate constant k_{ij} can be determined by saturating site j and measuring both the loss of intensity at site i and the apparent spin–lattice relaxation time of site i. For some amides these techniques have been tested on the base-catalyzed change,[14] where neither intramolecular exchange nor E/Z isomerization intrudes, so that we may have confidence in our ability to separate the simultaneous exchange pathways.

Experimental Section

Materials. Amides, reagent grade solvents, and acids were commercial samples from Aldrich, Mallinckrodt, Sigma, or AR Bader, used without further purification. *N*-Methylcyanoformamide was prepared according to Persson and Sandström;[15] mp 74–5 °C (lit.[15,16] 80–81, 68 °C). Cyclohexanol was redistilled from CaO. Solutions for kinetic studies were prepared from weighed amounts of amide, to produce a solution near 1 M, plus a measured amount of acid or trichloroacetate buffer sufficient to produce exchange at a rate readily measurable by NMR techniques. Trace amounts of water added to the organic solvents were found to have negligible effect on rate ratios.

Instrumentation. FT–NMR spectra were obtained with a Varian HR220 spectrometer adapted for FT use or with a Nicolet 1180E computer interfaced to an Oxford 360-MHz magnet. Probe temperature was 22–23 °C. Spin–spin coupling constants were determined with a Varian EM390 spectrometer. Signal assignments for most of these amides have been made previously,[14] and others are by analogy; except for *N*-*tert*-butylformamide and formanilide, an *E* substituent is downfield of the *Z*. The equilibrium constant $K_e = [Z]/[E]$ was determined by integration.

Kinetic Methods. Some rate constants were obtained by digital equivalents of line-shape analysis. Simple equations[17] relate rate constants to extra or residual line broadening (H_E and CH of (*Z*)-formanilide). Alternatively, the valley-to-peak intensity ratio of CH doublets (*N*-methylenes of *N*-formylglycine, CH of (*E*)-formanilide, *N*-methyls of *N*-methylcyanoformamide) can be tabulated[18] as a function of the inherent line width and the rate constant. Rate constants determined from a spin-coupled doublet include a statistical factor of 2, since only half of the proton exchanges interchange α and β spin.

Some rate constants (k_{ZE} for *N*-methylformamide in ethylene glycol and k_{ES}, k_{ZS}, and k_{ZE} for *N*-*tert*-butylformamide) were determined by saturation-transfer experiments[9,10] that involve saturating one resonance and measuring the intensities at others, as well as measuring apparent spin–lattice relaxation times, T_1^{app}. For *N*-methylformamide the resonances were the *N*-methyl peaks, and the observed rate constant was divided by 2 to account for the fact that only half the E/Z isomerizations exchange protons. For *N*-*tert*-butylformamide the resonances were NH and OH. For *N*-methylformamide in both water and ethylene glycol it was also possible to determine the weighted sum, $(p_E k_{ES} + p_Z k_{ZS})/p_S$, where p_i is the relative population of the ith site, by saturating simultaneously both NH resonances and determining the intensity and apparent T_1 of solvent. For *N*-*tert*-butylformamide the value of k_{EZ} could be confirmed by analysis of the coalescence of the two *tert*-butyl singlets.

For the most complicated case, *N*-methylformamide, where the $^4J_{HCNCH}$ distorts the *Z*-methyl, a combination of saturation transfer and line-shape analysis was necessary to evaluate all rate constants. The line shape was simulated[19] according to eq 7 of ref 14 (misprinted: $+\mathbf{K}$ should be $-\mathbf{K}$), with the rate constant matrix **K** as given in eq 3. (The ijth element, $\mathbf{K}_{ij}$, is k_{ji}, the rate constant for exchange from site j to site i. Diagonal elements are omitted, but $\mathbf{K}_{ii} = -\sum_j \mathbf{K}_{ji}$.) The eight sites are, from low field to high, the doublet of doublets (J = 4.8, 0.5 Hz) for CH_{3E} and the doublet of doublets (J = 4.8, 0.8 Hz) for CH_{3Z}. The justification for this **K** is given in the Discussion. Values of spin–spin relaxation times were obtained from spectra obtained under nonexchange conditions. For studies in ethylene glycol, values of k_{ZE} and k_{EZ} ($= k_{ZE}/K_e \approx k_{ZE}/13.5$) from saturation-transfer measurements were input and values of k_{ES} and k_{ZS} were adjusted until the simulated spectrum matched the observed one. For studies in aqueous solution, it was convenient to utilize the simplification that the simulated line shape in the slow exchange limit (before coalescence) depends chiefly on the diagonal elements of **K** and is nearly independent of the partitioning of that sum down a column. Thus comparison of simulated and observed line shapes provided values for $^1/_2k_{ZS} + {}^3/_2k_{ZE}$ and $^1/_2k_{ES} + {}^3/_2k_{EZ}$. These, along with the weighted sum, above, as well as the relative populations from solution composition or spectrum integration, then permitted evaluation of k_{ZS}, k_{ES}, and k_{ZE}. This latter method was also applied in ethylene glycol and found to give the same value for k_{EZ} as was determined by the former, direct saturation-transfer method. Further details are available.[20]

Results

Second-order rate constants for acid-catalyzed proton exchange of formamides are collected in Table I, along with the equilibrium constant $K_e = [Z]/[E]$. Values for $k_{ZE}/[H^+]$ are specific for acid-catalyzed exchange, since the contribution due to uncatalyzed rotation about the C–N bond was measured independently under nonexchange conditions and subtracted from the rate constant observed under the conditions of Table I. This procedure also corrects for any dipole–dipole relaxation.[9b] Error analysis, based on replicability of the NMR data plus propagation of errors, indicates that these values have a precision of ±10%. The value obtained for $k_{ES}/[H^+]$ of *N*-methylformamide in aqueous solution agrees very well with a published value[6a] of 22.6 M^{-1} s^{-1}.

The data show that there are two classes of amides, depending on whether rates of intermolecular proton exchange and E/Z isomerization are similar. This conclusion is also quite apparent from qualitative inspection of NMR spectra. Only the last three amides show coalescence at intermediate acidities to separate singlets for each stereoisomer. This behavior corresponds to rapid proton exchange and slow isomerization, as was observed[14] in the base-catalyzed exchange of three of these amides. In contrast, the behaviors of *N*-methylformamide and *N*-*tert*-butylformamide differ qualitatively between base and acid, since only in acid do the N-alkyl signals coalesce with each other.

Discussion

Kinetics Expected for the N-Protonation Mechanism. Scheme I shows the complete N-protonation mechanism for proton exchange in a secondary amide. The amide is present as an equilibrium mixture of (*E*) and (*Z*) stereoisomers (**2E** and **2Z**), whose pseudo-first-order rate constants of protonation are $k_{(E)}$ and $k_{(Z)}$, respectively. Protonation by H_S^+ from solvent initially produces one of the three conformers (**3**–**5**) shown for the intermediate, $RCONH_2^+R'$, or else its enantiomeric conformer. The choice of these rotamers as the stable conformers is by analogy

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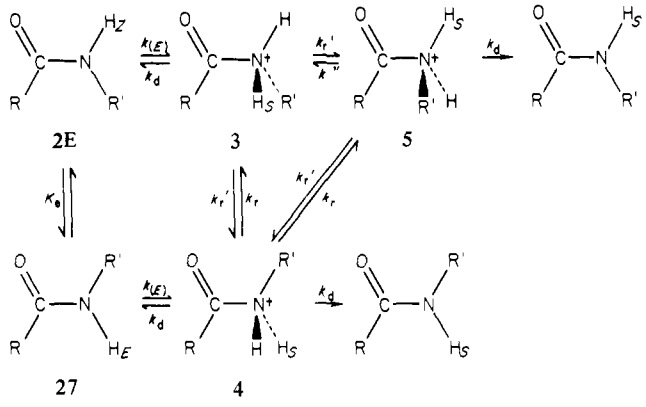
Scheme I. N-Protonation Mechanism for Exchange in (*E*) and (*Z*) RCONHR', Including Competition between Deprotonation and Rotation about the C-N Single Bond in the Intermediate, $RCONH_2^+R'$



to threefold rotors on an sp^2 carbon,[21] where a substituent eclipses the double-bonded group. Inasmuch as the barriers to rotation about sp^2–sp^3 single bonds are low, other rotamers might be involved, although strictly they cannot be intermediates. Such possibilities have been considered[6d] and rejected previously,[8b] as well as possibilities involving concerted rotation and protonation, so for simplicity we will not consider them here.

For generality, Scheme I allows for competition between deprotonation of the intermediate $RCONH_2^+R'$ and rotation about its C–N single bond. No assumptions are made about relative rates of conformational interconversion, except those required by symmetry. However, the same rate constant, k_d, regardless of conformation, is assumed for deprotonation, since this is a diffusion-controlled process[22] and ought to be the same for any acidic proton. In contrast, a proton eclipsing the oxygen is too weakly acidic to be removed at a competitive rate, so k_d refers only to skew hydrogens.

Scheme I leads to the matrix **K** used in line-shape analysis of *N*-methylformamide. It sufficies to show how three of the matrix elements are evaluated; the remainder are analogous. The matrix element $\mathbf{K}_{71}$ is the rate constant for exchange from the lowest-field *N*-methyl peak to the peak corresponding to the (*Z*) isomer with the same CH spin but opposite NH spin. In Scheme I this exchange occurs by isomerizing the (*E*) amide to (*Z*) concomitant with proton exchange, via conformers **3** and **4** (and possibly **5**). Also, since the two protons are equivalent in **3**, isomerization with proton exchange occurs at the same rate as isomerization without proton exchange, for which the rate constant is defined as k_{ZE}. However, only half the proton exchanges reverse the proton spin, so $\mathbf{K}_{71} = {}^1/_2k_{ZE}$. The matrix element $\mathbf{K}_{51}$ corresponds to isomerizing the (*E*) amide to (*Z*) while preserving the NH spin. There are two ways to achieve this: isomerization without proton exchange, with rate constant k_{ZE}, and isomerization with proton exchange, with rate constant ${}^1/_2k_{ZE}$, since both isomerization processes occur at the same rate but only half the solvent protons introduced have the same spin as the original NH. Therefore $\mathbf{K}_{71}$ is the sum of these two terms, or ${}^3/_2k_{ZE}$. The matrix element $\mathbf{K}_{31}$ is the rate constant for NH spin change of the (*E*) isomer without isomerization. In Scheme I this process occurs by transferring a proton from the H_Z site of **2E** to solvent, via conformer **5**. The rate constant for proton exchange in **2E** is k_{ZS}, but some of these proton exchanges, with rate constant k_{ZE}, also isomerize the amide, so this must be subtracted. Again, only half the proton exchanges reverse the NH spin, so $\mathbf{K}_{31} = {}^1/_2(k_{ZS} - k_{ZE})$.

Solution of Scheme I ($R' \neq H$) by the steady-state approximation, applied to the three intermediates (**3**–**5**), leads to the site-to-site rate constants in eq 4–6, along with eq 7, as required

$$\mathbf{K} = \begin{vmatrix}
- & 0 & {}^1/_2(k_{ZS} - k_{ZE}) & 0 & {}^3/_2k_{EZ} & 0 & {}^1/_2k_{EZ} & 0 \\
0 & - & 0 & {}^1/_2(k_{ZS} - k_{ZE}) & 0 & {}^3/_2k_{EZ} & 0 & {}^1/_2k_{EZ} \\
{}^1/_2(k_{ZS} - k_{ZE}) & 0 & - & 0 & {}^1/_2k_{EZ} & 0 & {}^3/_2k_{EZ} & 0 \\
0 & {}^1/_2(k_{ZS} - k_{ZE}) & 0 & - & 0 & {}^1/_2k_{EZ} & 0 & {}^1/_2(k_{ES} - k_{EZ}) \\
{}^3/_2k_{ZE} & 0 & {}^1/_2k_{ZE} & 0 & - & 0 & {}^1/_2(k_{ES} - k_{EZ}) & 0 \\
0 & {}^3/_2k_{ZE} & 0 & {}^1/_2k_{ZE} & 0 & - & 0 & {}^1/_2(k_{ES} - k_{EZ}) \\
{}^1/_2k_{ZE} & 0 & {}^3/_2k_{ZE} & 0 & {}^1/_2(k_{ES} - k_{EZ}) & 0 & - & 0 \\
0 & {}^1/_2k_{ZE} & 0 & {}^3/_2k_{ZE} & 0 & {}^1/_2(k_{ES} - k_{EZ}) & 0 & -
\end{vmatrix} \quad (3)$$

Table I. Rate Constants, in M^{-1} s^{-1}, for Acid-Catalyzed Proton Exchange in RCONHR' at 22 °C

amide	R	R'	[Z]/[E]	$[H^+]$, M	$k_{ES}/[H^+]$	$k_{ZS}/[H^+]$	$k_{ZE}/[H^+]$	$K_eK_{ES}/k_{ZE} - 1$
formamide-d_1[a]	D	H	1	0.014[b]	105	84	74[c]	
N-methylformamide	H	CH_3	13.5	0.14	26	56	13	26
				0.07[b]	39	150	28	18
N-*tert*-butylformamide	H	$C(CH_3)_3$	2.2	0.03,[b] 0.15[b]	13	15	10	1.8
N-formylglycine	H	CH_2COOH	12	7.9, 0.64, 334[d]	2.2	28	0.01	2600
formanilide	H	C_6H_5	2.6	0.7[e]	8	54	<<1	>>20
N-methylcyanoformamide	NC	CH_3	18	3.5, 1.1, 1.7×10^4[d]	1.8	16	$<10^{-4}$	$>3 \times 10^5$

[a] From ref 10b. [b] In ethylene glycol. [c] $k_{EZ}/[H^+] = 73$ M^{-1} s^{-1} by independent measurement. [d] Acidity function, $h_0 = 10^{-H_0}$. [e] In cyclohexanol.

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$$k_{ES} = \tfrac{1}{2}k_{(Z)} \quad (4)$$

$$k_{ZS} = \tfrac{1}{2}k_{(E)}\frac{k_r' + 2k_r''}{k_d + k_r' + 2k_r''} \quad (5)$$

$$k_{ZE} = \tfrac{1}{2}k_{(E)}\frac{k_r'}{k_d + k_r + k_r'} \quad (6)$$

$$K_e = \frac{[Z]}{[E]} = \frac{k_{(E)}k_r'}{k_{(Z)}k_r} \quad (7)$$

by detailed balance. These equations have the proper limiting behavior, such that if deprotonation is much faster than rotation, only H_E of the (Z) amide will exchange. The quantities on the left in eq 4–7 represent experimentally determined data in Table I. Unfortunately, there are only four equations in the six unknowns, $k_{(E)}$, $k_{(Z)}$, k_d, k_r, k_r', and k_r'', and there are no additional independent data possible. It is certainly not possible to solve for the unknowns. It is not possible even to solve for all the ratios of the unknowns. Therefore we are faced with a set of underdetermined equations, and there are so many consistent solutions that it is impossible mathematically to reject Scheme I. However, it is possible to consider more qualitatively whether the observed data are consistent with this scheme and with ideas of molecular structure.

Comparison of Observed and Expected Kinetics. We wish to demonstrate that the results in Table I are not quite consistent with eq 4–7, derived from Scheme I. Even though it is not possible to solve for all the ratios of rate constants, it is possible to derive eq 8. Values of this quantity are also tabulated in Table I. The

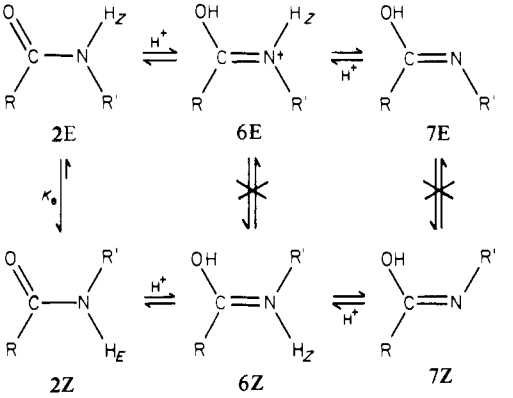
$$\frac{k_d + k_r'}{k_r} = \frac{K_e k_{ES}}{k_{ZE}} - 1 \quad (8)$$

data show that there are two classes of amides, depending on whether this quantity is "large". Owing to the factor of k_{ZE} in the denominator, the set of three amides for which this quantity is "large"—*N*-formylglycine, formanilide, and *N*-methylcyanoformamide—is just those amides for which E/Z isomerization is significantly slower than intermolecular proton exchange.

Mathematically it is possible that $(k_d + k_r')/k_r \gg 1$, either because $k_d/k_r \gg 1$ or because $k_r'/k_r \gg 1$, but this is not chemically likely. The ratio k_r'/k_r is the equilibrium constant between the anti conformer (**4**) and one gauche conformer (**3** or **5**) of $HCONH_2{}^+R'$. In isoelectronic aldehydes, $HCOCH_2R'$, this ratio has been observed[23] to be 3.7 (R = Me), 0.41 (R = *t*-Bu), and 1.8 (R = Ph) at 36 °C in acetonitrile, and it has been calculated[24] to be 3.2 (R = Me). Replacement of CH_2 by $NH_2{}^+$ is unlikely to affect these ratios substantially, since it has little effect on the rotational barrier in $HCOCH_3$,[25] and the steric interactions should be quite similar, since the C–C and C–N^+ bond lengths are similar. Even solvation should not affect the conformational preference greatly. Therefore a very large $(k_d + k_r')/k_r$ cannot be due to a large k_r'/k_r but would require a large k_d/k_r.

Is it reasonable that $k_d \gg k_r$ for the latter three amides in Table I? In $RCONH_3{}^+$ k_d and k_r are competitive,[10b] but both are exceedingly fast, since k_d represents a diffusion-controlled deprotonation by solvent and k_r represents a rotation with a very low barrier. In $RCONH_2{}^+R'$ the deprotonation is still diffusion-controlled and the rotational barrier is still low.[24] Besides, there is no reason why k_d would be much greater than k_r for these amides but not for N-*tert*-butylformamide. Even though these amides have electron-withdrawing groups, so that the deprotonation is more favorable thermodynamically, it is not possible to increase k_d, which is already at its diffusion-controlled limit.[22] Therefore we conclude that k_d also is not much greater than k_r, that $k_d + k_r' \gg k_r$ is not reasonable on chemical grounds, and that Scheme I does not account for the observed kinetics of *N*-formylglycine, formanilide, or *N*-methylcyanoformamide.

Scheme II. Imidic Acid Mechanism for Proton Exchange in a Secondary Amide



Even for *N*-methylformamide and *N*-*tert*-butylformamide there are difficulties in reconciling the data to Scheme I and eq 4–7. For *N*-*tert*-butylformamide there is no solution to the underdetermined equations that has all rate constants positive, although this could be due merely to the experimental errors in the data. For *N*-methylformamide there are many solutions to the equations, but none of them show $k_r' > k_r'' > k_r$, as calculated[24] for the isoelectronic $HCOCH_2Me$, and there is no set of $k_r{:}k_r'{:}k_r''$ ratios common to both solvents. Besides, all of these solutions require k_d/k_r to be at least 16, and we have claimed above that such values are unreasonably high. Moreover, the solutions to the equations for *N*-*tert*-butylformamide require $k_d/k_r \sim 1$. Such a small ratio was observed for some $RCONH_3{}^+$, but it is unlikely that *N*-*tert*-butylformamide would resemble primary amides more than *N*-methylformamide in this regard or in mechanism. None of these discrepancies alone is sufficient to disprove Scheme I, but together they certainly indicate that it is inadequate to account for the details of the observed results.

Imidic Acid Mechanism. Since Scheme I does not satisfactorily account for the data in Table I, we turn to an alternative mechanism. Scheme II shows the imidic acid mechanism for proton exchange in a secondary amide. This is considerably simpler than Scheme I, since E/Z interconversion in both intermediates (**6** and **7**) is too slow to contribute. The increased double-bond character in the conjugate acid (**6**) raises the barrier to rotation.[26] The lifetime of the imidic acid (**7**) is too short—ca. 10^{-9} s at pH 1, owing to encounter-controlled[22] reprotonation—to permit inversion.[27]

This imidic acid mechanism readily accounts for the exchange behavior of *N*-formylglycine, formanilide, and *N*-methylcyanoformamide. The large values of $K_e k_{ES}/k_{ZE}$ are not due to large values of $(k_d + k_r')/k_r$ in Scheme I, but to the absence of acid-catalyzed isomerization in Scheme II. The small k_{ZE} observed for *N*-formylglycine shows though that the N-protonation mechanism does contribute slightly, to the extent of ca. 1% of the total. Similarly, the discrepancies between Scheme I and the rate constants observed for *N*-methylformamide and *N*-*tert*-butylformamide can be ascribed to an incursion of the imidic acid mechanism. This is not the dominant mechanism for these two amides, since k_{ZE} is appreciable. Unfortunately, the data do not permit an assessment of the relative contribution of the two mechanisms.

Stereochemistry and Substituent Effects. For the three amides that exchange predominantly by the imidic acid, k_{ZS} is appreciably greater than k_{ES}. This suggests that the (E) stereoisomer of the imidic acid (**7**) is more stable than the (Z), as has been observed for imidate esters[28] and calculated for formimidic acid[5] and methyl

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formimidate.[29] However, it is necessary to take into account the relative stabilities of the reactant (*E*) and (*Z*) amides. If reprotonation rate constants are the same for both **7E** and **7Z**, as is reasonable[30] for an encounter-controlled reaction of two so similar substrates, then K_e^{1A}, the equilibrium constant for the imidic acids, is given by eq 9. Values of this equilibrium constant

$$K_e^{1A} = \frac{[7Z]}{[7E]} = K_e\frac{k_{ES}}{k_{ZS}} \qquad (9)$$

are 0.9 (*N*-carboxymethylformimidic acid), 0.4 (*N*-phenylformimidic acid), and 2.0 (*N*-methylcyanoformimidic acid). Thus the (*E*) imidic acid is not always the more stable one. The stability difference is quite small, since whatever stabilizes the *Z* amide[31] seems to persist in the imidic acid. Besides, both dipole–dipole interactions and solvation are likely to affect the relative stabilities, as in some primary imidic acids, RC(OH)=NH, where the (*Z*) form is more stable.[10b]

The changeover in mechanism is readily understood in terms of transition-state structure. Both reactions are acid-catalyzed, so both will be retarded by electron-withdrawing substituents, as is observed in Table I. For both mechanisms the reverse of the rate-limiting step is a thermodynamically favorable proton transfer, so that the transition states resemble[32] the intermediates $RCONH_2^+R'$ and RC(OH)=NR'^+. Since the former transition state bears a greater positive charge, it will be more strongly destabilized by electron-withdrawing substituents. Therefore such substituents as *N*-carboxymethyl, *N*-phenyl, and α-cyano strongly retard the N-protonation mechanism and by default favor the imidic acid mechanism. Similar substituent effects were seen in *N*-methylacetamides.[12]

Relative to primary amides, secondary amides seem more likely to exchange via the imidic acid. For example, the data in Table I indicate that, within experimental error, formamide exchanges nearly exclusively via N-protonation, whereas we have concluded that *N*-methylformamide and *N-tert*-butylformamide exchange partly via the imidic acid. Similar mechanistic shifts have been seen with acetamides.[12] This is opposite to what is expected from an electron-donating alkyl group. However, the effect of *N*-alkyl can be seen in Table I to be slightly retarding, and we attribute these effects to a steric hindrance to solvation of $RCONH_2^+R'$.

Finally, it remains to justify the use of the acidity function[33] H_0 in calculating some of the second-order rate constants in Table I. The transition state for the N-protonation mechanisms resembles $RCONH_2^+R'$, which in turn resembles a protonated aniline, for which the extent of protonation is governed by H_0. Therefore, this acidity function is a reasonable approximation to the acidity function that governs the rate of N-protonation. In contrast, the imidic acid mechanism requires water as a base for proton removal from the conjugate acid (**6**) of the amide. Although formation of this conjugate acid is governed by an acidity function H_A,[34] the requirement of water[35] and the resemblance of the transition state to the imidic acid suggest that excess acidity[36] does not promote the reaction and that $[H^+]$ itself governs the rate of the acid-catalyzed imidic acid mechanism. It should be noted that even though mechanisms were implicitly assumed in presenting and then interpreting the data in Table I, the reasoning is not circular, since the use of alternative measures of acidity would not affect the mechanistic conclusions.

Conclusions

Secondary formamides with electron-withdrawing substituents exchange predominantly by the imidic acid mechanism. In particular, this conclusion applies to *N*-formylglycine, our closest model for a peptide linkage in a protein, and the contribution of the N-protonation mechanism is less than 1% of the total. Therefore we reject the mechanism for acid-catalyzed proton exchange in proteins that had been accepted for more than 20 years. Secondary formamides with simple alkyl substituents on nitrogen exchange by a combination of the two mechanisms. This conclusion is different from that for formamide itself or for *N*-alkylacetamides, where the N-protonation mechanism predominates, but it can be rationalized in terms of substituent effects and steric hindrance to solvation. The question of how general these conclusions are, and whether less polar solvents might change them, is considered in the accompanying publication.

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Registry No. 1 (R = D), 35692-88-7; **2** (R = H; R′ = Me), 123-39-7; **2** (R = H; R′ = *t*-Bu), 2425-74-3; *N*-formylglycine, 2491-15-8; formanilide, 103-70-8; *N*-methylcyanoformamide, 39088-41-0.

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