Proton Exchange in Amides: Surprises from Simple Systems

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The Problem

For many years biochemists have been studying proton exchange (eq 1) in amides, peptides, and proteins (**1**).[1] They observed that protons buried in the

$$\underset{\mathbf{1}}{\mathrm{RCONHR'}} + \mathrm{H_2O} \rightleftharpoons \mathrm{RCONHR'} + \mathit{H}\mathrm{OH} \quad (1)$$

interior of a protein are slow to exchange. By measuring rates, they could count the number of buried protons, information also available by X-ray diffraction. They could also learn about the dynamics of protein motion—how those buried protons escape into solvent or how solvent gains access to them. This is information not available by X-ray diffraction.

The earliest studies were done by letting deuterium or tritium wash into or out of the protein and analyzing for isotopic content. Then in 1959 some physical chemists showed that NMR spectroscopy offers a more elegant method.[2] The adjacent alkyl group R′ is split into a doublet by spin–spin coupling with the NH proton. As that proton exchanges, the doublet broadens, coalesces, and resharpens. Line-shape analysis then provides the exchange rate constant directly from a single spectrum, rather than by plotting the time dependence of isotopic content.

The reaction is both base- and acid-catalyzed. There is no question about the mechanism of the base-catalyzed exchange. Hydroxide removes the NH proton (eq 2), to produce the imidate anion (**2**), which is subsequently reprotonated. (We have recently succeeded

$$\mathrm{RCONHR'} + \mathrm{OH^-} \rightleftharpoons \underset{\mathbf{2}}{\mathrm{RC(O^-){=}NR'}} + \mathrm{H_2O} \quad (2)$$

in preparing imidate anions and characterizing them and their E/Z stereoisomerization by NMR.[3]) The mechanism of the acid-catalyzed exchange had always been accepted as involving N-protonation (eq 3). This is analogous to the base-catalyzed exchange, except that the sequence of deprotonation and protonation is reversed.

$$\mathrm{RCONHR'} + \mathrm{H^+} \rightleftharpoons \mathrm{RCONH_2{}^+R'} \quad (3)$$

Yet it was always biochemists and physical chemists who were studying this reaction, never an organic chemist. An organic chemist knows better, or at least I thought I did. Everyone agrees that the most basic site of an amide is not the nitrogen but the oxygen. Protonation on oxygen then acidifies the NH proton for water to remove it (eq 4) and produce the imidic acid

$$\mathrm{RCONHR'} + \mathrm{H^+} \rightleftharpoons \mathrm{RC(OH){=}NHR'^+} \rightleftharpoons \underset{\mathbf{3}}{\mathrm{RC(OH){=}NR'}} + \mathrm{H^+} \quad (4)$$

Orginally from Pittsburgh, Charles L. Perrin graduated from Harvard College in 1959 and received his Ph.D. in 1963 from Harvard University, under the direction of F. H. Westheimer. Following postdoctoral research at U. C. Berkeley, he joined the founders of the new campus at U. C. San Diego. He has been a Special NIH Research Fellow at Gothenburg University and a NATO Visiting Professor at the University of Padua. His research interests span a broad range of physical–organic chemistry.

(3), which returns to amide by reversing the steps. Although more circuitous, this mechanism seemed more attractive, since it avoids protonating on nitrogen, which is not very basic.

Since biochemists continue to use hydrogen exchange to probe protein structure and protein dynamics,[4] it is worth elucidating the mechanism. Besides, the imidic acid (**3**) is the abnormal tautomer of an amide and is also of interest. The preference expressed for the N-protonation mechanism (eq 3) was not a trivial oversight. The imidic acid mechanism (eq 4) had been considered and rejected,[1c,5] but for questionable reasons. On the other hand, the N-protonation mechanism is not completely impossible, since tertiary amides, RCONR′R″, show an acid-catalyzed isomerization,[2,6] which can occur only by N-protonation. By comparing the rates of these two processes, Martin[7] had concluded that the imidic acid mechanism can be operative or dominant. We sought decisive evidence.

Distinguishing the Mechanisms

It is not obvious how to distinguish the two acid-catalyzed mechanisms, since they are so similar. Indeed, one group[8] had despaired of doing so. Yet we have succeeded, with several surprises along the way. Although we had thought that this would be a straightforward project, it has led to important and fundamental results of more significance than just the question of mechanism.

Our initial approach[9] was to abandon the adjacent alkyl group and turn to primary amides (**4**). These have

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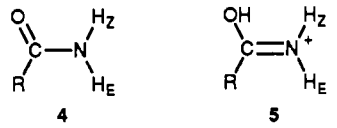
protons, H_E and H_Z, in two different environments,



4 **5**

distinguishable by NMR spectroscopy. Even upon O-protonation, to form **5**, they are still in different environments, and they ought to exchange at different rates. In contrast, in the N-protonated intermediate, $RCONH_3^+$, those two protons have become equivalent, and they would necessarily exchange at identical rates. Thus there is a simple distinction between the two mechanisms. Either the two protons exchange at different rates, or they do not. Besides, we can use the base-catalyzed exchange to assess how different those rates might be, since H_Z and H_E in **4** should also be removed by OH^- at different rates.

Since this is such a simple system, previous studies could suggest what we might expect. MO calculations[10] on the parent formimidic acid indicated that the *E* configuration (**6E**, R = H) is more stable than the *Z* (**6Z**). Likewise, NMR and dipole moment studies[11] on

6E **6Z** **7Z** **7E**

imidate esters (N-substituted **6**, R = alkyl) showed that the *E* configuration is favored. For comparison, MO calculations[12] on imidate anions suggest that the *Z* configuration (**7Z**) is more stable than the *E* (**7E**), and this is consistent with experimental results on the isoelectronic carboxylic acids and esters.[13] From elementary thermodynamics it then follows that H_Z is the more acidic NH proton in **5**, but H_E is the more acidic in **4**. From Eigen's results,[14] kinetic acidity of NH protons should assuredly parallel thermodynamic acidity. Therefore we expected H_E to exchange faster in base, but H_Z to exchange faster in acid. The crossover arises because stabilities of the imidic acids (**6**) are determined by dipole–dipole interactions, whereas stabilities of the imidate anions (**7**) are determined by lone-pair repulsions, and these happen to behave differently.

The reason no one had done such kinetic experiments previously is that the ^{14}N quadrupole broadens NH protons nearly to invisibility. However, we could decouple ^{14}N electronically. Subsequently we discovered that viscous solvents also effectively decouple ^{14}N, and ethylene glycol and sulfuric acid are viscous enough. By either of these methods the NH peaks are sharpened. Peak widths are still ca. 5 Hz, but the two NH peaks

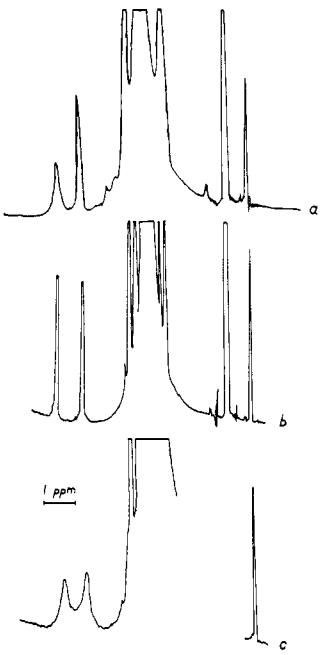


Figure 1. ^{14}N-Decoupled 100-MHz ^1H NMR spectrum of 0.7 M aqueous acetamide: (a) pH 7.94; (b) pH 5.95; (c) pH 1.94. Peaks, from left to right, are H_E, H_Z, H_2O (with spinning side bands), CH_3, and internal *tert*-butyl alcohol. Reprinted with permission from ref 9. Copyright 1974 American Chemical Society.

Table I
Relative Rates of Base-Catalyzed and Acid-Catalyzed Exchange of H_E and H_Z in Aqueous $RCONH_2$

R	k_E^{OH}/k_Z^{OH}	k_E^{H}/k_Z^{H}
CH_3	7.5 ± 0.9	1.17 ± 0.02
CH_2=CH	4.0 ± 0.4	1.49 ± 0.04
CH_2=$C(CH_3)$	1.75 ± 0.02	2.5 ± 0.4
$(CH_3)_3C$[a]	1.5 ± 0.3	1.10 ± 0.06
$NCCH_2$	3.6 ± 0.6	1.25 ± 0.19
CF_3	2.1 ± 0.25	1.61 ± 0.06
Ph[a]	3.6	1.97 ± 0.38

[a] Aqueous methanol.

are separated by 0.3–1.0 ppm, so that their individual exchange behavior can be readily determined by line broadening. Besides, in some special cases, we have used ^{15}N-labeled amides, but these are not generally necessary. Peak assignments are no problem. Generally, the downfield proton of an amide is H_E.[15] In a few cases where there was uncertainty, new assignments were made.

Immediate Surprises

Figure 1b shows the ^1H NMR spectrum of aqueous acetamide, CH_3CONH_2, at pH 5.95.[9] At this pH, proton exchange by any mechanism is too slow to detect. Figure 1a shows the NMR spectrum at pH 7.94, where the base-catalyzed exchange proceeds rapidly enough to broaden the NH peaks. It is obvious that H_E, the downfield peak, is broader. It is exchanging faster, exactly as expected. The two NH environments are not merely abstractly different, but detectably so by kinetics.

Figure 1c shows the NMR spectrum of aqueous acetamide at pH 1.94. The solution contains predominantly CH_3CONH_2, with very little of other forms. The low pH just "tickles" the solution and catalyzes the

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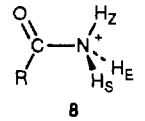
exchange so that the NH peaks broaden. *They broaden to different extents*! Admittedly, the difference is not obvious. It is more obvious that the heights of the NH peaks differ, and since they must have identical areas, they indeed have different widths and therefore different rates. In this case the rate difference is only 17%, but the effect is general. Table I shows rate ratios for both base- and acid-catalyzed exchange for a series of amides.

It is quite clear that the two NH protons do not become equivalent. I was delighted! I had disproved the N-protonation mechanism (eq 3). I had proved the imidic acid mechanism (eq 4). I was writing a manuscript for publication, but there was one annoying discrepancy. *The wrong proton exchanges faster.* It is H_E that exchanges faster, not only in base, where it was expected, but also in acid, where H_Z should be faster. Indeed, in ethyl acetimidate, $CH_3C(OEt)=NH_2^+$, it *is* H_Z that exchanges faster.[16] Therefore the results are not consistent with the imidic acid mechanism either.

When *all* possible mechanisms are eliminated, it is a sign that an assumption, often implicit, is unwarranted. Above, it was asserted that the NH protons of the N-protonated intermediate, $RCONH_3^+$, become equivalent. Of course they become equivalent. They are like the hydrogens of a methyl group, which we always treat as equivalent. All it takes to make them equivalent is rotation about a C–N or C–C single bond. That is an sp^2–sp^3 single bond, with a barrier to rotation of only ca. 1.0 kcal/mol,[17] corresponding to a rate of rotation above 10^{12} s^{-1}. (Such a value has been measured for the methyl rotor in acetone.[18]) Ordinarily that rate is so high that we can automatically assume that CH_3 hydrogens or NH_3^+ protons are equivalent. But among the processes that might be comparably fast is proton transfer. The intermediate $RCONH_3^+$ is an exceedingly strong acid. Its pK_a has been estimated[19] as ca. −8. Proton transfer to H_2O is thus quite exergonic, and such a proton transfer is expected[14] to be diffusion-controlled. Water is the solvent. It does not need to diffuse to the $RCONH_3^+$. It is already there, solvating those acidic protons. The simplest model for a diffusion-controlled reaction with solvent is to multiply the second-order rate constant[14] of 2×10^{10} M^{-1} s^{-1} by 55 M, the concentration of water, to obtain a rate constant of 10^{12} s^{-1} for deprotonation of $RCONH_3^+$. Despite the uncertainties, this is so close to the rate of C–N rotation that we are not justified in assuming rapid rotation or equivalent protons.

We must look more closely at the N-protonation. According to MO calculations,[20] the preferred conformation of the N-protonated intermediate is 8, with a barrier to rotation still ca. 1 kcal/mol. The proton



8

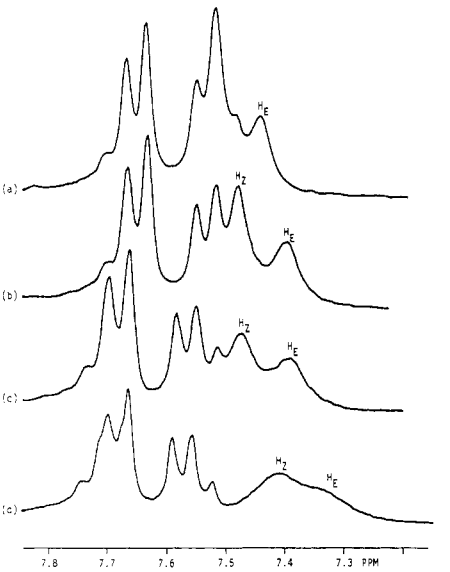


Figure 2. NMR spectrum (downfield region) of benzamidine in aqueous H_2SO_4: (a) 75 wt %; (b) 77 wt %; (c) 79 wt %; (d) 82 wt %. Reprinted with permission from ref 22b. Copyright 1980 American Chemical Society.

labeled H_Z in 8 could not have entered from solvent. That would require rotation about the C–N bond of the amide, and that rotation is known to be much too slow to account for the observed exchange. Likewise, rotation did not transfer the H_E proton of the amide (4) to the site labeled H_Z in 8. The proton in that site must be the original H_Z proton of the amide. The solvent proton, H_S, therefore must have entered nearly perpendicular to the molecular plane, which is where the nitrogen lone pair is. This produces conformer 8 (plus its enantiomer), with the labeling as shown. What can 8 do next? It can lose H_S and revert to the original amide. It can lose H_E, whereby H_E has exchanged with solvent. It cannot lose H_Z, by microscopic reversibility! Since H_Z did not enter from solvent, it cannot be lost to solvent. (Alternatively, loss of H_Z would create a twisted amide, lacking amide resonance.) In order for H_Z to exchange, there must be rotation about the C–N bond of 8. If that rotation is fast, relative to deprotonation, we return to the naive assumption: The three protons would be equivalent, and H_E and H_Z would exchange at identical rates. If rotation is slow, relative to deprotonation, only H_E can exchange. If rotation and deprotonation are competitive, H_E will exchange faster than H_Z. That *is* what we observed. It was not considered to be fully consistent with the imidic acid mechanism (eq 4). It *is* consistent with the N-protonation mechanism (eq 3) after all.

Independent Tests

If this mechanism is correct, it is a remarkable one. It requires the rate-limiting step for H_Z exchange to be rotation about the C–N single bond, even though that step has a rate constant ca. 10^{12} s^{-1}. The opportunity to probe such fast processes makes this reaction more important than just a question of mechanism. Therefore it is necessary to obtain further evidence.

Amidinium ions (9) provide a suitable test. These had been observed[21] to undergo acid-catalyzed proton

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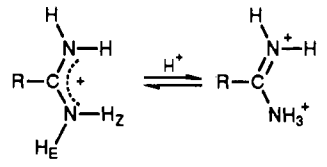
exchange in H_2SO_4, for which N-protonation represents the only reasonable mechanism (eq 5). Naively, we



(5)

9

would have expected the protons of the NH_3^+ group to have become equivalent, so that H_E and H_Z would exchange at identical rates. That is not observed! Instead H_E exchanges detectably faster than H_Z (Figure 2).[22] The NH protons do not become equivalent, and rotation about the C–N single bond can be rate-limiting.

Is this really the mechanism for amides? We have rejected the imidic acid mechanism (eq 4) on flimsy evidence and dubious reasoning. The evidence is merely a slight extra broadening of H_E relative to H_Z (Figure 1, Table I), both of which are already broadened by the ^{14}N quadrupole. The reasoning relies on previous studies[10,11] of **6E** and **6Z**, but those studies were for vacuum or nonpolar solvents. The dipole–dipole interactions that determine relative stabilities ought not be so effective in water, and the stabilities may reverse. Therefore we seek a new approach to the question of mechanism.

There is another distinction between the two mechanisms. The imidic acid mechanism (eq 4) is simple, in that the two protons exchange independently with H_2O. The N-protonation mechanism (eq 3) is more complicated, in that it also allows for intramolecular exchange. Above, it was noted that exchange of H_Z in **8** requires rotation about the C–N bond. Even after rotation, H_Z is not necessarily lost to H_2O. Instead it may only be transferred to the H_E site. It can readily be shown that the N-protonation mechanism leads to eq 6, where k_{ij} is the pseudo-first-order rate constant

$$k_{EZ} = k_{ZE} = k_{ZS} \leq k_{ES} \quad (6)$$

for exchange from site i to site j. The simplicity of the imidic acid mechanism (and the slowness of sp^2 nitrogen inversion, compared to the short lifetime of the imidic acid) means that $k_{EZ} = 0 = k_{ZE}$, just as in the base-catalyzed exchange (which served as a test system for all the new NMR methodology). Therefore we must determine whether there is acid-catalyzed intramolecular proton exchange equally as fast as the intermolecular exchange.

That is easy to say but not so easy to do. The usual NMR line shape methods give the lifetime of a proton in a given site, say H_Z. The reciprocal of that lifetime is the sum $k_{ZS} + k_{ZE}$, but we need to evaluate those two rate constants separately.

New NMR Techniques for Chemical Kinetics

Saturation-transfer methods are capable of separating such rate constants. Most previous studies[23] had relied on the ability of saturation transfer to study two-site exchange reactions on a slower time scale than the usual NMR methods. The unique advantage of saturation transfer is that it provides site-to-site rate constants in multisite systems. Often these are decisive in deriving mechanistic information. The experiment is the same as a nuclear Overhauser enhancement study, and it is easy with Fourier-transfer instrumentation. One site is irradiated, to equalize its populations of α and β nuclear spin states. Only the excess of spins in the lower energy state can produce a net absorption signal. When that excess is destroyed, the signal disappears. It is said to be saturated. If nuclei are exchanging from that site into another, that other site will also be saturated and its signal will diminish. (Maybe this should be called a nuclear Underhauser effect.) The saturation is not necessarily complete, since spin–lattice relaxation restores the excess. One can measure each diminution in intensity (eq 7, where $I_i(j)$, i and j = E, Z, and S, is

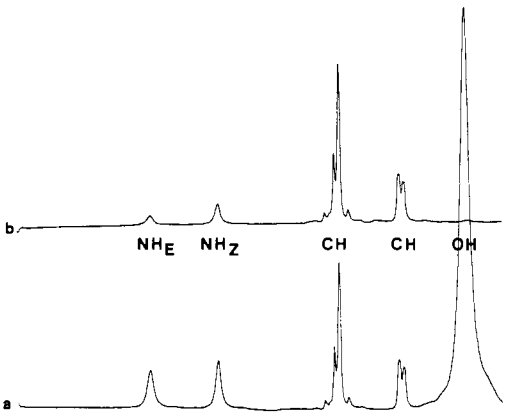


Figure 3. Saturation-transfer experiment on 2.5 M acrylamide in ethylene glycol at apparent pH 8.21 (plot width 900 Hz (solvent CH_2 off scale to right)): (a) off-resonance spectrum; (b) with saturation of solvent OH. Reprinted with permission from ref 28. Copyright 1981 American Chemical Society.

$$t_i(j) = [I_i^\circ - I_i(j)]/I_i^\circ \quad (7)$$

the signal intensity of site i when site j is saturated and I_i° is its equilibrium intensity without saturation) and each apparent spin–lattice relaxation time, $T_{1i}(j,k)$, of site i when sites j and k are saturated. It is then possible to evaluate all six site-to-site rate constants in a three-site system according to eq 8.[24]

$$k_{ij} = [t_i(j) - t_i(k)t_k(j)]/T_{1i}(j,k)[1 - t_j(k)t_k(j)] \quad (8)$$

Quantitative two-dimensional (2D) exchange NMR offers an alternative to saturation transfer. The pulse sequence is 90°–t_1–90°–t_m–90°–t_2(acquire), where t_m is a mixing time during which chemical exchange can occur. The signal is subjected to a double Fourier transform with respect to both the incremented variable t_1 and the usual t_2. The result is a 2D spectrum with a normal spectrum along the diagonal and with cross peaks corresponding to site-to-site exchange. Early examples[25] were qualitative, to map out exchange pathways. With proper phase cycling,[26] to obtain absorption-mode spectra in both dimensions, it is possible to obtain reliable signal intensities and to convert those by matrix algebra to all the site-to-site rate constants in a multisite system.[27]

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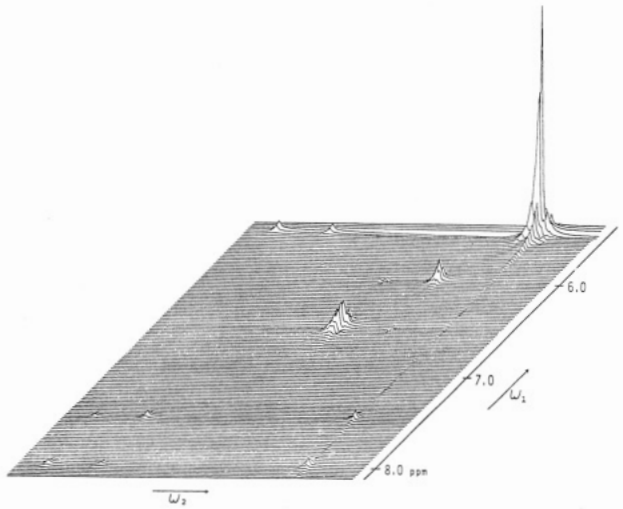

Figure 4. Two-dimensional pure-absorption-phase spectrum of acrylamide in ethylene glycol at apparent pH 1.75. The peaks along the diagonal, from bottom left to top right, are H_E, H_Z, CH (vinylics), CH (vinylic), and H_S (OH of solvent); CH_2 of solvent is off scale at lower ω_1 and ω_2. Reprinted with permission from ref 27. Copyright 1984 American Chemical Society.

Figure 3 shows saturation-transfer ^1H NMR spectra of base-catalyzed NH exchange in acrylamide, $CH_2{=}CHCONH_2$. The transfer of saturation from OH to NH can be seen to be greater for H_E, which is exchanging faster. Figure 4 shows a 2D NMR spectrum of acid-catalyzed NH exchange in acrylamide. Not only are there cross peaks between NH and OH but also there are peaks between NH_E and NH_Z, demonstrating intramolecular exchange.

Evaluation of site-to-site rate constants[27,28] from spectra like Figures 3 and 4 (and subtracting the contribution to k_{EZ} and k_{ZE} from uncatalyzed C–N rotation, measured independently) shows that eq 2 is satisfied, within experimental error, for six different primary amides. I must admit that those biochemists and physical chemists were correct all along. These amides do exchange by the N-protonation mechanism (eq 3). Despite my intuition, O-protonation does not acidify the NH proton enough for water to remove it, and exchange requires direct protonation on nitrogen, even though this occurs rarely.

Furthermore, for each of these amides (as well as for some amidinium ions[22b]), H_E is observed to exchange faster than H_Z. Thus this was not some artifact of the line-broadening method,[9] since it is also observed by saturation transfer and 2D exchange NMR. As proposed above, the intermediate $RCONH_3^+$ is so strong an acid that it does not live long enough to achieve rotational equilibration about its C–N single bond. In further support of this interpretation, it was observed[16] that $k_{ZE} = k_{ZS} = k_{ES}$ in concentrated H_2SO_4, since $RCONH_3^+$ lives longer in this less basic medium.

There is also a class of amides for which eq 6 is not satisified. For amides such as ethyl oxamate, $EtOCOCONH_2$, intramolecular exchange is significantly slower than intermolecular.[28] Such behavior is inconsistent with the N-protonation mechanism. At last I had found evidence for the imidic acid mechanism (eq 4). It is not general, though, but applies only to amides with electron-withdrawing substituents. (Amides with weakly electron withdrawing substituents, such as malonamide, $H_2NCOCH_2CONH_2$, exchange by a mixture of the two mechanisms.) The changeover of mechanism may be rationalized in terms of transition-state structures. Both mechanisms involve endergonic proton transfer in the rate-limiting step. Then according to Hammond's postulate,[29] the transition state for the N-protonation mechanism resembles the N-protonated intermediate, whereas the transition state for the imidic acid mechanism resembles the imidic acid. Both these reactions are acid-catalyzed, so both are retarded by electron-withdrawing substituents, as had long been recognized.[5] However, the transition state for the N-protonation mechanism bears a larger positive charge, so it will be even more strongly destabilized by electron-withdrawing substituents, which thus favor the imidic acid mechanism.

Secondary Amides

Who cares about primary amides? Except for some side chains,[30] peptide and protein NH protons are in secondary amides (**10**). The comparison (eq 6) of intermolecular with intramolecular proton exchange in primary amides here becomes the comparison of proton exchange with Z/E isomerization (eq 9), since only

10Z **10E** (9)

N-protonation, and not the imidic acid mechanism, can interconvert these stereoisomers. These rates can be measured by a combination of line-shape analysis and saturation-transfer studies.[31] For three amides, $HCONHCH_2COOH$, HCONHPh, and $NCCONHCH_3$, there is only proton exchange, without isomerization. These exchange by the imidic acid mechanism, as expected from the electron-withdrawing substituents. For three other amides, $HCONHCH_3$, $HCONHC(CH_3)_3$, and $F_3CCH_2OCONHCH_3$, isomerization accompanies proton exchange. With these electron-donating substituents, the N-protonation mechanism intrudes.

Yet these few amides are not of biochemical interest. They are merely the ones that are amenable to the desired comparison, which requires a sufficient concentration of the minor stereoisomer, **10E**. The R substituent of biochemically interesting amides is too bulky, so they are almost exclusively **10Z**.

Fortunately the results on the primary amides suggested that a study of inductive effects in secondary amides might be informative. Accordingly we returned to old-fashioned line-shape analysis of NH proton exchange in aqueous *N*-methylacetamides, $XCH_2CONHCH_3$.[32] (Restriction to acetamides was necessary, in order to hold steric and resonance effects constant.) Figure 5 shows a plot of the second-order rate constant for acid-catalyzed proton exchange vs a

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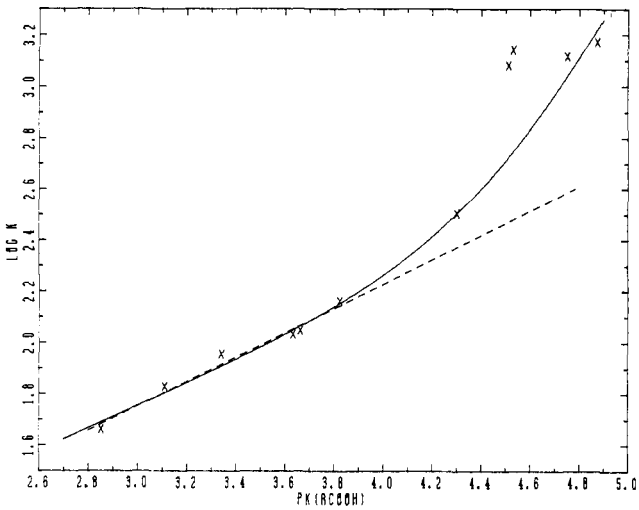
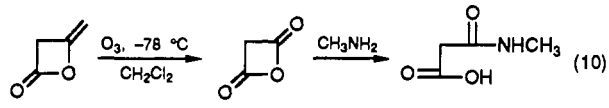


Figure 5. Correlation between log k_{H^+} for acid-catalyzed proton exchange in *N*-methylacetamides, $XCH_2CONHCH_3$, and the pK_a of the corresponding XCH_2COOH: dashed line, best linear fit for six amides, slope = 0.48; solid curve, $k_{H^+} = 10^{0.43pK_a+0.46} + 10^{1.84pK_a-5.88}$. Reprinted with permission from ref 32. Copyright 1982 American Chemical Society.

measure of the inductive effect of X, namely, the pK_a of XCH_2COOH. For six such amides with electron-withdrawing X = HO, I, etc., the points fall nicely on a straight line with slope 0.48. That represents a reaction that is not very sensitive to substituent effects, as expected for a mechanism whose transition state resembles the imidic acid. Incidentally, one of those points is X = HOCO, which was synthesized (eq 10) via



(10)

11

malonic anhydride (11), a simple molecule that had eluded synthesis for 80 years until we prepared it.[33] The remaining points in Figure 5 deviate positively. For electron-donating X = H, CH_3, Ph, $HOCOCH_2$, and $CH_3NHCOCH_2$, another mechanism intrudes. This mechanism is much more sensitive to substituent effects (slope ca. 1.8), as expected for a mechanism whose transition state resembles the N-protonated intermediate. Thus, by the criterion of substituent effects we see the same (gradual) changeover of mechanism with secondary amides as we saw with primary amides by the comparison of intramolecular and intermolecular exchange. Despite the electron-donating *N*-alkyl group, secondary amides are slightly more likely to exchange by the imidic acid mechanism, and this can be rationalized.[31]

Tergiversation

What about X = RCONH, which is the substituent in peptides and proteins? That is the fifth point from the left in Figure 5. As might be guessed by comparison with the other substituents, RCONH is an electron-withdrawing group. *Therefore the NH protons of peptides and proteins exchange predominantly by the imidic acid mechanism (eq 4), not by the previously accepted N-protonation mechanism!* Besides, peptides and proteins have an additional electron-withdrawing

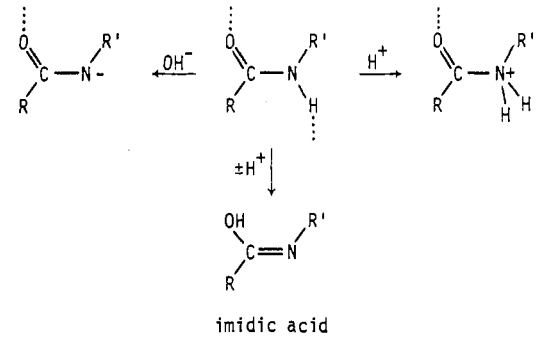


Figure 6. Comparison of accessibility requirements of base-catalyzed, N-protonation, and imidic acid mechanisms for NH exchange in the interior of proteins. The dotted lines represent internal hydrogen bonds that may need to be broken to permit the exchange. Reprinted with permission from ref 34. Copyright 1984 American Chemical Society.

CH_2CONHR' in place of the CH_3 of Figure 5, and the evidence from $HCONHCH_2COOH$ above shows that this further favors the imidic acid mechanism.

Since the mechanism is so sensitive to substituent effects, might it also be sensitive to solvent effects? The NH protons buried in the interior of a protein may be in a nonpolar environment. Must the imidic acid mechanism still predominate? To answer this question, we studied two amides, CH_3CONH_2 and $HCONHCH_3$, in a series of solvents.[34] As solvent polarity was reduced from water or ethylene glycol to cyclohexanol–dioxane or aqueous THF, the extent of intramolecular exchange or *E*/*Z* isomerization accompanying acid-catalyzed NH exchange decreased, especially for the secondary amide. Therefore the imidic acid mechanism becomes even more predominant in less polar solvents. We may estimate that the N-protonation mechanism contributes only ca. 0.1% to the overall NH exchange in peptides and proteins.

This conclusion has important implications for the mechanism whereby solvent gains access to buried protons. Two extremes have been proposed—solvent penetration by small-amplitude protein motions or a local unfolding of the protein. While the N-protonation mechanism was accepted, the acid- and base-catalyzed reactions seemed similar, in that only the nitrogen would need to be accessible to solvent (Figure 6). However, to create the imidic acid, the oxygen must also be accessible, as has been realized independently by Tüchsen and Woodward.[35] Moreover, the imidic acid reverses the hydrogen-bond donor–acceptor properties of the amide (Figure 6). Therefore both the oxygen and the nitrogen must be dislodged from their original environment as amide—α-helix or β-sheet. The necessity of exposing the entire amide fragment to solvent then suggests that a partial unfolding is involved.

A corollary of the substituent effects is that electron donation leads to rapid acid-catalyzed proton exchange in ureas, but by the N-protonation mechanism. In particular, biotin (**12**, R = $HOCO(CH_2)_4$) undergoes acid-catalyzed exchange via **13**, rather than via the isourea **14**,[36] which is formed too slowly to account for enzymatic carboxylation of biotin. Although the isourea seemed like a reasonable intermediate, the intermediate that accepts the CO_2 is probably the ureide, **15**, formed

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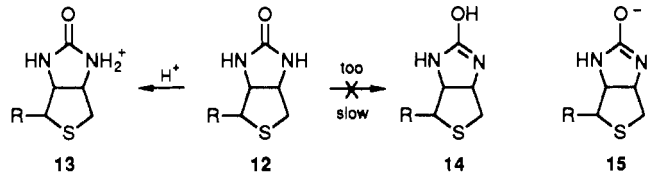


13 12 14 15

by base-catalyzed deprotonation.

Rotation of Hydrogen-Bonded NH_3^+

Initially we had assumed that rotation about the C–N single bond of $RCONH_3^+$ is extremely fast. Then we realized that deprotonation is competitive. We estimated rate constants for both these processes at 10^{12} s^{-1}. Is this correct? Although exergonic NH proton transfers are generally diffusion-controlled, this one resembles deprotonation of $RCOCH_3$, which is retarded by the need for solvent reorganization or electron delocalization.[37] Also, the MO calculations[20] that justified rapid rotation do not take account of solvation. Since the NH protons are very acidic, the NH_3^+ must be strongly hydrogen bonded to solvent. Rotation of the NH_3^+ requires breaking and remaking three hydrogen bonds.

We need an independent estimate of the rate of that rotation. We cannot study $RCONH_3^+$ itself, since amides are O-protonated. Fortunately the simplest analogue, NH_4^+, is amenable to study, and this fundamental ion is of interest in its own right. It too might be strongly fixed within its solvation shell, since the enthalpy of hydration is 10.6–20.6 kcal/mol per water molecule[38] and since MO calculations on $NH_4^+ \cdot OH_2$ suggest that 5.5 kcal/mol is required to bifurcate each hydrogen bond.[39]

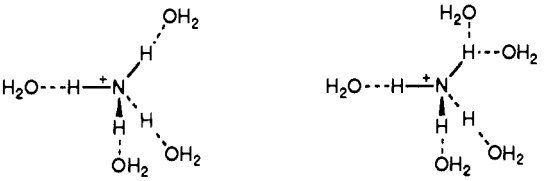
We can use ^{15}N NMR spectroscopy, since ^{15}N spin–lattice relaxation in $^{15}NH_4^+$ is governed by the motion of the attached protons, as the ion tumbles within its solvation shell. (Nuclear Overhauser enhancements verify that only the attached protons contribute, and not those in solution.) From the spin–lattice relaxation time T_1 we can determine the rotational correlation time, τ_c, which is the average time required for the NH_4^+ to rotate by 34° about any axis. For aqueous NH_4^+, τ_c is only 1.1×10^{-12} s![40] This is remarkably fast, faster than a water molecule rotates and almost as fast as the 0.2×10^{-12} s for CH_4 in liquid CH_4,[41] which definitely is not hydrogen-bonded. It is too fast for rotation of an $NH_4^+(OH_2)_4$ unit, so the NH_4^+ must be breaking its hydrogen bonds as it rotates. Nor is the NH_4^+ rotating by tunneling, since ND_4^+ rotation is retarded only by its $2^{1/2}$-fold increased moment of inertia. Indeed, the observed τ_c corresponds to a barrier to rotation of only 1.6 kcal/mol, far less than the energy required to break (even partially) three hydrogen bonds.

To understand why the rotation is so fast, we studied it in a series of solvents.[42] Water seems unusual, and rotation in other solvents is slower, with τ_c up to 20×10^{-12} s (Table II). The rates do not parallel solvent polarity, solvent dielectric-relaxation time, solvent viscosity, the ability of the solvent to accept a hydrogen

Table II
Rotational Correlation Times of the Ammonium Ion

solvent	T_1, obsd (s)	τ_c (ps)
96% H_2SO_4	57	0.46
water	44	1.1
water-d_2 (ND_4^+)	409	1.5
85% H_3PO_4	16.6	1.7
18-crown-6/acetone	16.4	2.8
50% v/v aqueous ethanol	18	2.8
methanol	13.6	3.8
ethylene glycol	8.5	6
pyridine	4.97	10
glycerol	3.6	12
dimethyl sulfoxide	3.96	13
ethanol	3.6	14
N-methylacetamide	2.6	20

bond, or the dipole moment of the solvent molecule. What is special about water is its molecular size. Although NH_4^+ fits into the ice lattice,[43] with four directional hydrogen bonds (**16a**), aqueous NH_4^+ is presumably more disordered, with more than four water molecules in its first solvation shell (**16b**). We surmise[42] that additional solvent molecules facilitate rotation because only a single hydrogen bond needs to be broken, and the new hydrogen bond can be made after only a small displacement. Indeed, the other media that show short τ_c are H_2SO_4, H_3PO_4, and 18-crown-6, all of which can cluster many hydrogen-bond-acceptor oxygens around the NH_4^+.



16a 16b

These data provide a bonus concerning diffusion-controlled reactions. Above we have estimated a rate constant of 10^{12} s^{-1} for a diffusion-controlled reaction where one reactant is the solvent. This estimate is quite uncertain. However, we now have the rate of rotation of aqueous NH_4^+, which can be used to estimate how solvation reduces the calculated[17,20] rate of rotation of $RCONH_3^+$. Also, from the relative reactivities of H_E and H_Z, we can evaluate the relative rates of rotation and deprotonation of $RCONH_3^+$. We can thereby estimate[44] a rate constant of 6×10^{10} s^{-1} for deprotonation of the strong acid $RCONH_3^+$ by surrounding solvent water. This is slightly lower than the previous estimate, and it may be that the need for solvent reorganization or electron delocalization retards this deprotonation. It would be interesting to compare this rate with the lifetime of a sufficiently acidic excited-state naphthol in water.

Summary

Acid-catalyzed NH proton exchange in amides RCONHR′ can proceed via the imidic acid RC(OH)=NR′ or via the N-protonated intermediate $RCONH_2^+R'$. By various NMR techniques, both old—line-broadening and line-shape analysis, substituent effects on rates—and new—saturation transfer, quantitative 2D exchange

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NMR—the mechanisms could be distinguished. It was concluded that amides with electron-donating substituents exchange by N-protonation, whereas amides with electron-withdrawing substituents exchange via the imidic acid. This latter class includes peptides and proteins, and the implications for solvent accessibility to buried NH protons and for carboxylation of biotin have been discussed. The N-protonation mechanism, in both amides and amidinium ions, shows the novel feature that the intermediate is so strong an acid that it does not live long enough to achieve rotational equilibration about its C–N single bond. Rotation of solvated NH_3^+ is hardly restricted by hydrogen bonding to solvent, as judged from the rotational correlation time of aqueous NH_4^+, which is only 1.1×10^{-12} s. Rotation is considered to be so fast because of multiple coordination of solvent molecules to the hydrogen-bonded protons.

I am grateful to my co-workers, cited in the references, for their excellent skills. This research has been supported by continuing grants from NSF, following an initial grant from Research Corporation.

Synthesis of Polycyclics via Aryne Arylation Reactions

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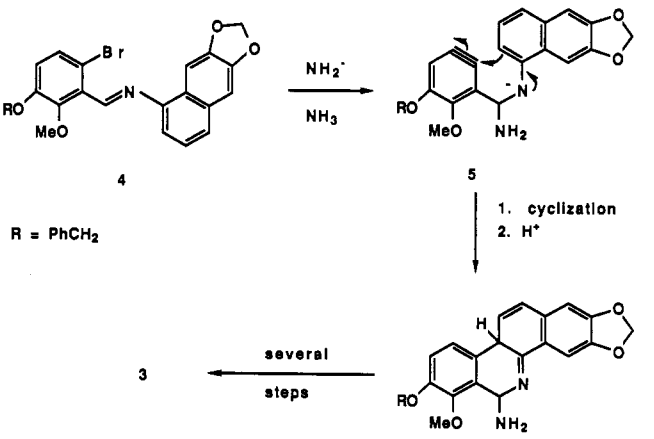
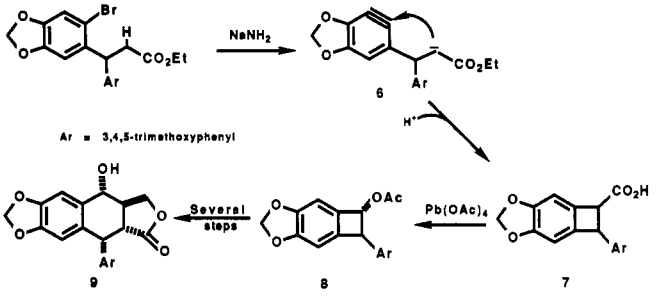
The benzyne molecule was proposed and supported by Wittig[1] as early as 1942 to explain the remarkable ease with which nonactivated haloarenes undero nucleophilic substitution in the presence of a strong base. The intermediacy of benzynes in such reactions was shown conclusively by J. D. Roberts in 1953.[2] Since then, this substance and its derivatives have proven to be potent tools in synthetic design.[3] Fueling the dramatic rise of the use of these reactive intermediates have been the high electrophilicity and dienophilicity of their bent acetylenic bond enforced by the geometry of the benzene ring.[4] These two chemical properties have been exploited in many synthetic strategies, the most notable being the construction of rings (i.e., annulation[5]) onto the highly reactive triple bond of the benzyne. The annulations have been carried out in two general ways. One capitalizes on the superb dienophilic properties of benzyne by treating arynes with dienes to yield polycyclic compounds such as iptycenes,[6] condensed polynuclears,[7] novel rings,[8] and certain natural products.[7,9] The other utilizes the electrophilicity of benzyne by adding appropriately substituted nucleophiles to arynes in an initial step followed by cyclization. These arylations may proceed either intramolecularly or intermolecularly. In the former case, termed "benzyne cyclization", both the ring fusion and the addition of an appropriate side chain occur simultaneously, whereas in the latter case, the initially formed adduct is subsequently cyclized in situ or after suitable structural modification.

Scheme I



Scheme II



This Account will focus on the application of these aryne arylations to the synthesis of polycyclic com-

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Subhash P. Khanapure received his Ph.D. degree from Poona University under the supervision of Dr. K. G. Das at the National Chemical Laboratory, Pune, and Regional Research Laboratory, Hyderabad. After spending one and one-half years working as a postdoctoral fellow in Prof. K. Fuji's laboratory in the Institute for Chemical Research at Kyoto University, he joined Ed Biehl's research group at Southern Methodist University in 1985. His research interests are in the area of synthetic organic chemistry.

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