Nuclear Magnetic Resonance Study of Amide Monomers in a Polypeptide Helix–Random Coil Interconverting Media*

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ABSTRACT: Proton high-resolution nuclear magnetic resonance spectra of *N*-methylacetamide and *N*,*N*-dimethylacetamide have been examined in mixtures of trifluoroacetic acid and chloroform (a solvent system, when appropriately constituted, that is postulated to support helical–random coil or interconverting structural forms of certain polypeptides) in order to contrast the spectral behavior of simple amides with that of appropriate polypeptides. Proton spectral displacements for the acid proton of trifluoroacetic acid, N-methyl protons, the amide proton, and C-methyl protons are interpreted in terms of equilibria involving the amide, an ion pair consisting of the conjugate acid of the amide and the trifluoroacetate ion, and the dissociated ion pair. Concentrations of the various species are functions of the per cent acid; amide is present in absence of acid, the ion pair is maximized at very nearly 1:1 acid–amide, and the ion pair completely dissociates as the acid is increased. Kinetic processes, amide–proton exchange, and rotation about the amide–carbon bond are shown to behave consistently with these structural interpretations.

Cooperative structural transitions of model polypeptides in dilute solution have been shown to be effected at room temperature in specific ranges of trifluoroacetic acid (TFA)[1]–chloroform ($CHCl_3$) mixtures (Fasman, 1963; Glick *et al.*, 1966; Stewart *et al.*, 1967). The ordered or helical configuration is stable at intermediate and high chloroform concentrations while the disordered structure is favored in high acid regions. In general, the stability of either form is postulated to be dependent on a balance between intramolecular and intermolecular interactions involving the amide functional groups of the polypeptide and, when appropriate, the solvent. It has been recently demonstrated that proton nmr techniques can be used to study intra- and intermolecular effects contributing to configurational stability of polypeptides (Glick *et al.*, 1966; Stewart *et al.*, 1967). These polypeptide nmr studies prompted an investigation of proton nmr spectral features of simple amides in this polypeptide-interconverting media in order to determine aspects of the amide–solvent interaction uncomplicated by polypeptide chain effects. The study includes not only an examination of proton nmr spectral features of *N*-methylacetamide (NMA) and *N*,*N*-dimethylacetamide (DMA) in the solvent system TFA–$CDCl_3$, but also the exchange rate of the amide proton of NMA as well as rotation about the CN bond in DMA.

Experimental Section

The nmr measurements were made with a Varian Associates HR60 spectrometer at a constant temperature of 30°. The peak positions were measured from tetramethylsilane as internal standard using the usual side-band technique. For each peak calibration 6–10 sweeps were averaged. The peak positions are accurate to ± 0.2 cycle/sec.

The amides were obtained from Eastman Organic Chemicals and were refluxed over anhydrous sodium sulfate and distilled through a glass, helix-packed column before use. The trifluoroacetic acid, obtained from Matheson Coleman and Bell, was distilled at atmospheric pressure before use and stored in a dry box in an all-glass container. The amide solutions were made up by weight under anhydrous conditions.

Proton Chemical Shifts

A single, sharp proton nmr resonance peak is observed for the acid proton of TFA in mixtures of TFA–$CDCl_3$. The peak is displaced downfield by more than 2 ppm in the range 1–15 wt % acid; from 15 to 100 wt % acid very little change in the spectral position is noted. This spectral behavior corresponds to that observed for other carboxylic acids interpreted in terms of association through intermolecular hydrogen bonds (Reeves, 1961). The acid proton displacement is significantly changed upon the addition of a fixed concentration (about 1.9 M) of either NMA or DMA to the mixed solvent. In the presence of either amide, the acid peak in the low acid region is displaced more than 4 ppm below that for the comparable solvent system in the absence of amide. This behavior is fol-

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* From the Department of Chemistry and Institute of Molecular Biophysics, The Florida State University, Tallahassee, Florida. *Received July 15, 1966.* This work was supported in part by the U. S. Public Health Service Research Grants GM 11337 and GM 10614 and in part by a contract between the Division of Biology and Medicine, U. S. Atomic Energy Commission, and the Florida State University.

[1] Abbreviations used: $CDCl_3$, chloroform-*d*; DMA, *N*,*N*-dimethylacetamide; NMA, *N*-methylacetamide; nmr, nuclear magnetic resonance; TFA, trifluoroacetic acid.

TABLE I: Chemical Shifts and Exchange Rates of NMA Protons in TFA–$CHCl_3$ Mixtures.

Wt % TFA	NMA Concn (mole/l.)	Mole Ratio NMA:TFA	δ^a TFA	δ^a NH	δ^a CCH_3	δ^a NCH_3	J'^b	$\tau \times 10^2$ sec
0.00	1.88	—	—	7.127	1.988	2.730 2.810	4.8	
0.85	1.91	22	10.743	7.210	1.998	2.743 2.817	4.6	16.0
2.30	1.90	7.3	13.373	7.325	2.017	2.753 2.818	3.9	8.0
4.45	1.93	3.9	14.677	7.540	2.042	2.770 2.828	3.5	6.8
5.98	1.96	2.9	14.827	7.635	2.057	2.784 2.825	2.5	5.4
9.8	1.96	1.79	15.323			2.815		4.7
10.8	1.92	1.57	15.675	7.798	2.112	2.847		4.7
12.1	1.91	1.40	16.182	7.738	2.112	2.842		4.7
16.4	1.92	1.04	15.580	7.910	2.160	2.847 2.898	3.1	6.1
42.6	2.19	0.50	14.598	8.098	2.280	2.972 3.018	2.8	5.80
100.0	1.67	0.14	12.602	8.713	2.490	3.115 3.172	3.4	6.61

[a] Downfield chemical shifts in parts per million from tetramethylsilane. [b] Spin–spin splitting in cycles per second.

TABLE II: Chemical Shifts of TFA and DMA Protons in TFA–$CDCl_3$ Mixtures.

Wt % TFA	DMA Concn (moles/l.)	Mole Ratio DMA:TFA	δ^a TFA	δ^a CCH_3	δ^a NCH_3
0.0	1.90	—	—	2.077	2.932 3.032
6.38	1.93	2.84	15.152	2.118	2.967 3.060
12.53	1.92	1.43	15.912	2.167	3.142
21.20	2.03	0.90	16.158	2.230	3.083
100.0	1.94	0.15	12.897	2.540	3.333

[a] Downfield chemical shifts in parts per million from tetramethylsilane.

lowed by an upfield displacement to a position, in concentrated acid, approximately 1 ppm below that of the acid proton in the amide free solution. These observations are summarized in Figure 1 and Tables I and II. Parallel spectral displacements for the acid proton were observed in TFA–$CDCl_3$ solutions containing one-eighth the concentration of NMA as was used in the experiment described above, the maximum displacement, however, occurred at a lower acid fraction.

In all cases the maximum spectral displacement occurred at an acid:amide ratio very nearly unity. This strongly suggested a 1:1 amide–acid interaction. Analysis of the magnitude of the displacement indicated that the structure for this complex probably involves an associated ion pair, B, rather than a dissociated ion pair, C, as the chemical shift for the ion, C1, is known to be quite different than that observed (see Chart I) (Gillespie and Birchall, 1963). Furthermore, the magnitude supports a structure involving hydrogen bonding after proton transfer of the proton from the acid to the amide, B1, rather than a hydrogen bond interaction of the same general type postulated (Pople *et al.*, 1959) for the acid–acid association and should not result in a displacement significantly different from that found for the pure acid. An interaction of the

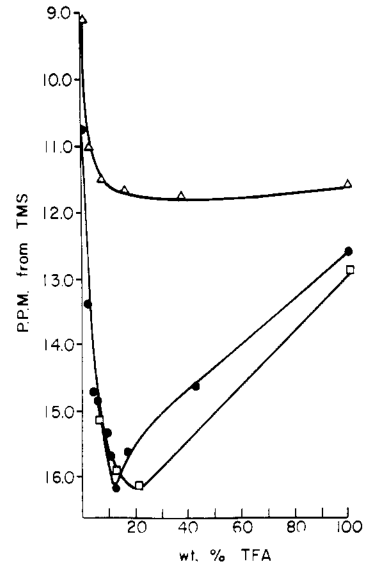


FIGURE 1: Proton nmr chemical shift of the acid proton or trifluoroacetic acid in TFA–CDCl₃ (△), TFA–CDCl₃–NMA (●), and TFA–CDCl₃–DMA solutions (□), in parts per million from tetramethylsilane. All shifts are downfield.

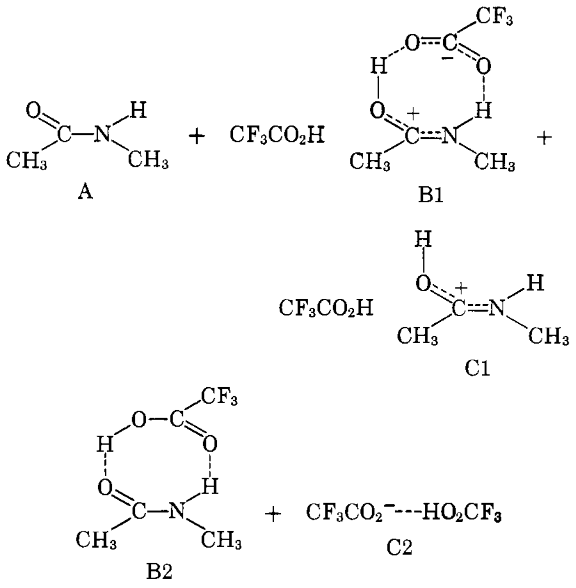


CHART I

B2 type is probably responsible for the fact that no acid proton nmr shift accompanies the dissolution of amides in acetic acid (Reeves, 1961).

We additionally postulate that above 16% acid, equilibria are displaced toward species C1 and C2. This is based on the analysis of displacements of the acid peak in the presence of the amide as a function of acid concentration. Thus, as only one peak is observed for the acid proton with, therefore, rapid exchange of that proton occurring between the TFA and the exchange sites in other structures, the position of the peak is given by

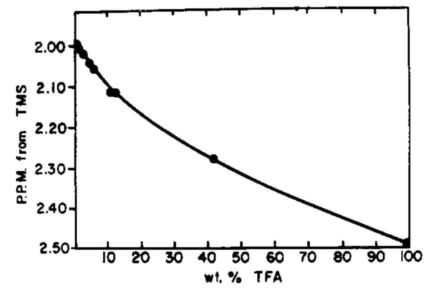


FIGURE 2: Proton nmr chemical shift in parts per million of the C-methyl of *N*-methylacetamide *vs.* weight per cent trifluoroacetic acid in a solution of TFA–CDCl₃. Shift is downfield.

$$\nu_{\text{obsd}} = \Sigma_i p_i \nu_i \qquad (1)$$

In eq 1, ν_{obsd} is the observed frequency, ν_i is the frequency of protons at the *i*th site, and p_i is the mole fraction of *i*th protons. Although eq 1 is not uniquely soluble, the observed chemical shift of the acid proton between 16 and 100% TFA can be minimally treated as the weighted average of the resonance frequencies for B1, uncomplexed TFA, and, as postulated, C1 and C2. In the absence of CDCl₃, eq 1 simplifies to

$$\nu_{\text{obsd}} = p_1 = \nu_1 + p_2\nu_2 + p_3\nu_3 \qquad (2)$$

In eq 2, experimental mole fractions are: $p_i = P_{\text{C1}} = 0.126$, $p_2 = P_{\text{C2}} = 0.126$, and $p_3 = P_{\text{TFA}} = 0.748$; $\nu_1 = -10.0$ ppm and $\nu_2 = (-20 \text{ ppm}) = \nu_3 = -11.7$ ppm. The value for ν_2 of -10 ppm was taken from Gillespie and Birchall's (1963) measurement of the chemical shift of the OH proton of protonated DMA at −80°. With this value for ν_1 and the experimental value for ν_3, ν_2 is found to be −21 ppm. The latter shift parallels that found for the acid proton when trifluoroacetate is added to TFA (Klotz *et al.*, 1964). In the region 16–42% acid, the shift data are compatible with p_1 varying from 1 to 0 and ν_2 of −19 ppm, in reasonable agreement with that given above.

The chemical shifts for NMA and DMA protons are also given in Tables I and II. In general, each type of amide proton is shifted downfield as the solvent composition is varied from 0 to 100% TFA, as would be expected upon carbonyl-oxygen protonation. A typical displacement, that given in Figure 2 for the C-methylamide protons, can be further decomposed into two regions corresponding to equilibria leading to the formation of B1 followed by the displacement toward C1. The rapidly varying solvent composition and high concentrations provide a poor basis for detailed thermodynamic elaboration.

Exchange of NH Proton of NMA

The resonance of the NCH₃ protons of NMA occurs as a doublet of varying separation throughout the entire range of TFA concentration; the separation disappears in the region from about 9–15% TFA with the peak resonance appearing as a broad singlet; it is assumed that the doublet collapse is due to the exchange of the amide and TFA protons. Thus, if in the absence of acid the lifetime of the amide proton is long, the lifetime, τ, is given by the relation (Reynolds and Schafer, 1963), separation of peaks/separation of peaks at large $\tau = [1 - (1/2\pi^2\tau^2(J_{\mathrm{HNCH_3}})^2)]^{1/2}$. In this expression $J_{\mathrm{HNCH_3}}$ is the coupling constant between the NH proton and the NCH_3 proton. In the regions where the doublet collapsed to a singlet, the relation (Reynolds and Schafer, 1963), $\tau = \sqrt{2}/2\pi J_{\mathrm{HNCH_3}}$, was used to obtain the lifetimes.

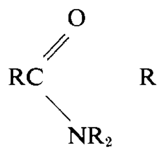
As may be seen from the data given in Table I, the lifetime of the NH proton decreases as TFA is added to the $CDCl_3$ solution of NMA until the TFA concentration reaches about 15%, after which the lifetime increases as more TFA is added. Thus, the maximum exchange rate occurs at the concentration at which the only ionic species in solution is the ion pair (B1) and unassociated amide. At TFA concentrations above 16% ion pair dissociation appears to correlate with a decreased exchange rate. These changes in proton exchange lifetimes appear to relate to the postulated changes in equilibria.

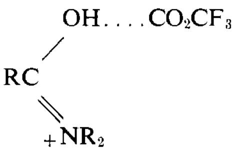
Rotational Freedom around the CN Bond in N,N-Dimethylacetamide

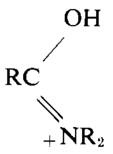
The signal for the N-methyl protons of DMA is a doublet below, and a singlet above, 12% TFA; this is attributed to the existence of a barrier to rotation around the CN bond lowered by acid catalysis. The coalescence of the doublet structure can be analyzed, using arguments similar to those given above, to obtain the lifetime for a configuration. What is significant is that the rotational barrier disappearance of DMA occurs at very nearly the same acid concentration as does the maximum exchange rate of amide–proton exchange in NMA; this suggests that the barrier height is decreased by a mechanism very similar to that for NH exchange. The mechanism for both processes may involve proton exchange between the oxygen and nitrogen of amide molecules; for DMA the barrier is destroyed by protonation of the nitrogen and loss of double bond character of the CN bond while for NMA, protonation forms an intermediate with equivalent amide hydrogens resulting in exchange upon deprotonation of the nitrogen. A similar mechanism has been postulated for barrier rotation catalysis in aqueous hydrochloric acid solutions (Berger *et al.*, 1959).

Conclusion

In presenting evidence that B1′ then C1′ represent structural changes experienced by an amide, A′,


A′


B1′


C1′

in solutions of increasing TFA concentration, we concur with similar suggestions derived from infrared and other studies (Klotz *et al.*, 1964; Hanlon *et al.*, 1963; Hanlon and Klotz, 1965; Hanlon, 1966); dynamic changes have been shown to amplify our conclusions. Without contradiction, therefore, all evidence reported to date supports the continuous acid-induced structural change for amides herein presented.

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