Proton Exchange of N-Methylacetamide in Concentrated Aqueous Electrolyte Solutions. I. Acid Catalysis

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Abstract: Acid-catalyzed amide hydrogen exchange kinetics of N-methylacetamide (NMA) have been measured in a variety of concentrated electrolyte solutions. Second-order rate constants for the exchange process (k), determined by nmr line-shape analyses, have been shown to be markedly dependent on the nature of the supporting cation, and quite insensitive (except for SCN^-) to the species of supporting anion present. Furthermore, it has been shown that log k is a linear function of supporting electrolyte concentration. A linear relationship has also been observed between log k and the reciprocal of the crystal radius of the supporting cation, suggesting that cationic charge density is an important factor in determining the exchange rate. The effects of added electrolyte on the rate constant do not seem to correlate with any of the usual solution electrolyte properties, but can be accounted for formally by postulating salt-induced changes in the activity of the transition-state complex. A mechanistic model is proposed involving a cation-dependent alteration in the equilibrium between the various tautomeric forms of protonated NMA, not all of which are active in the acid-catalyzed proton-exchange reaction. $^7Li^+$ nmr spin–lattice relaxation measurements performed in lithium halide mixed amide–water solutions revealed no anion dependence, within experimental error, of the spin–lattice relaxation time (T_1), confirming further that the interaction between the lithium ion and the amide dipole is not affected by the associated anion.

In addition to their nonspecific effects on electrostatic interactions, neutral salts exert striking and specific effects on the structure, conformational stability, and transconformation kinetics of a variety of macromolecules in aqueous solution.[1] However, the relative effectiveness of various salts in stabilizing (or destabilizing) macromolecular conformations with respect to transition to an unfolded form is quite independent of the chemical or conformational details of the macromolecules.[1] Both cations and anions (independently and additively) follow a general Hofmeister-type series in relative effectiveness as conformational perturbants, comparable to the series which have been demonstrated in the salting-out of simple nonpolar molecules from aqueous solution.[2] Results from a wide variety of experimental studies on model compounds suggest that these changes in macromolecular stability reflect changes in the free energy of transfer, from the nonaqueous interior of the macromolecule into the solvent milieu, of residues which become exposed as a consequence of the unfolding reaction.[3] Ionic additives can affect this free energy of transfer both indirectly, by modifying the structure of the aqueous solvent, and by direct ion–dipole interaction with polar groups such as the peptide (-NHC(=O)-) moiety which is characteristic of both proteins and nucleic acids. The present study of the interaction of ions with a simple amide was undertaken to illuminate further some aspects of the mechanism and specificity of the latter type of interaction.

The exchange kinetics of the amide hydrogens of both proteins and nucleic acids with solvent protons have recently been intensively studied in an attempt to obtain insight into the details of conformational mobility of various macromolecular structures,[4] and since concentrated electrolyte solutions can serve as very effective isothermal destabilizers of the "native" (folded) structures of macromolecules, hydrogen exchange studies of proteins and nucleic acids in concentrated salt solutions are currently of considerable interest.[5] Such studies are designed to determine how the rate and/or extent of exposure to solvent of various hydrogens "buried" in the interior of a macromolecule is altered by the addition of destabilizing salts to the aqueous environment, and are generally formulated in terms of a model which specifies that potentially exchangeable protons located in the interior of a macromolecule can only exchange with solvent when the conformation "opens" to expose these sites to the solvent. Schematically this process for a particular site may be written

$$C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} O \xrightarrow{k_3} \text{exchanged} \qquad (1)$$

where C and O represent the closed and open site, k_1 and k_2 are the rate constants for the opening $\rightleftarrows$ closing process, and k_3 is the rate constant for the chemical exchange process itself. In order to interpret the observed rates in terms of k_1 and k_2, it is necessary to know something about the effects of neutral salts on the intrinsic chemical exchange rate constant, k_3. Such information is currently not available.

To obtain such kinetic information, as well as to probe further the ion–amide interaction, we have used nuclear magnetic resonance techniques to examine the kinetics of the acid-catalyzed proton exchange of the simple amide, N-methylacetamide (NMA), in concentrated salt solutions. Similar studies have been reported for this amide in water,[6] and the mechanistic

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(2) F. A. Long and W. F. McDevit, *Chem. Rev.*, **51**, 119 (1952).

(3) D. R. Robinson and W. P. Jencks, *J. Amer. Chem. Soc.*, **87**, 2470 (1965); D. R. Robinson and M. E. Grant, *J. Biol. Chem.*, **241**, 4030 (1966); E. E. Schrier and E. B. Schrier, *J. Phys. Chem.*, **71**, 1851 (1967).

(4) K. Linderstrøm-Lang in "Symposium on Protein Structure," A. Neuberger, Ed., Metheun, London, 1958; A. Hivdt and S. O. Nielsen, *Advan. Protein Chem.*, **21**, 287 (1966); P. H. von Hippel and M. P. Printz, *Fed. Proc.*, **24**, 1458 (1965); S. W. Englander in "Biological Macromolecules," Vol. I, G. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 8.

(5) B. McConnell and P. H. von Hippel, in preparation.

interpretations previously advanced will be used as a framework for the current study. N-Methylacetamide is especially suited for a study of this type. Its nmr spectrum is relatively simple and sensitive to the mean time of proton residence on the amide nitrogen, its structure is characteristic of the amide linkages in proteins and nucleic acids, it does not form interpeptide hydrogen bonds readily in aqueous media,[7] and a wealth of proton exchange information (gained by both deuterium exchange and nmr techniques) exists in the literature.[8]

Materials and Methods

Chemicals. All inorganic chemicals used in this study were of reagent grade. The tetraalkylammonium salts (*e.g.*, TMACl, TEACl, etc.) were Eastman White Label grade and were used as received. The N-methylacetamide was also Eastman White Label, and was used without further purification since vapor phase chromatographic analysis (Wilkens A-700 Autoprep, 9-ft column of 20% Carbowax 20M at 180°) had revealed only a small amount of impurity, which was tentatively identified as methylamine. The nmr spectra of NMA, both in chloroform and water, showed only the expected features.[6] Hexamethyldisiloxane (HMDS) was purchased from K & K Laboratories (Plainview, N. Y.).

Preparation of Solutions. All solutions used in these studies were prepared in glass-distilled water, and were 1.0 *M* in amide and 2.78 *M* in salt (except LiCl, for which several salt concentrations were prepared). Usually each solution was filtered through a Whatman GF/A glass fiber filter. After adjustment to the desired pH with 6 *N* HCl, an aliquot was placed in an nmr tube and immediately sealed with a pressure cap (Wilmad Glass Co., Buena, N. J.). All solutions used in the rate studies were run on the nmr spectrometer within 6 hr after preparation.

Solutions used in the lithium ion relaxation study were prepared as above except that no pH adjustment was made and the concentrations of amide, salt, and water differed. For these studies 0.01 mol of lithium salt was dissolved in a total of 1.1 mol of solvent (amide + water) and the amide concentration was varied to give the following amide mole fractions: 0.091, 0.242, 0.343, and 0.495.

pH Adjustment and Measurement. A Radiometer Model 22 pH meter equipped with a Radiometer GK2021B combination glass–calomel electrode was used to measure the pH of the various aqueous salt–amide solutions. Titrations to the desired pH were carried out at 23 ± 1° in a jacketed reaction vessel equipped with either a nitrogen velocity barrier or a bubbler tube. The electrode was standardized against pH 1.00 and 4.00 Sørensen buffers (obtained from Brinkman, Westbury, N. Y.) and small corrections to the observed pH, when necessary, were made by linear interpolation. Control experiments indicated that the adjusted pH of the amide solutions was stable to ±0.05 pH unit for a period of at least 6 hr. This constancy of pH was also borne out by the fact that no changes with time were observed in the pH-dependent line shapes in any of the observed nmr spectra.

Large liquid junction potentials, leading to significant errors in pH determination (and hence in the calculated apparent proton activity), are not expected for concentrated aqueous salt solutions. Estimates of these potentials from data presented by Bates[9] indicate an upper limit of 2.6 mV which corresponds to a pH error of 0.04 pH unit at 25° (see also Rosenthal and Dwyer[10]). This has been confirmed by Nozaki and Tanford,[11] who estimated an error no greater than 0.02 pH unit for concentrated guanidinium hydrochloride solutions at this temperature. Thus in these studies, the observed (corrected) pH was set equal to the negative logarithm of the hydrogen ion activity ($-\log a_{H^+}$).

Viscosity Measurement. Solution viscosities were measured in simple Ostwald viscometers, suspended in a water bath maintained at 23 ± 0.02°. All out-flow times were measured six to ten times, using an electrical stopwatch which could be read to ±0.01 sec. A precision of better than 1% was obtained in these measurements. The solution viscosities were finally expressed as relative viscosities (out-flow times), referred either to pure water or to 1.0 *M* NMA in water, as appropriate.

Nmr Spectrometer Details. All proton spectra were recorded at 60 MHz using a Varian DA-60-IL spectrometer system at a probe temperature of 23 ± 1°. An external reference sample of HMDS held in a concentrically mounted capillary tube (Wilmad) was used to provide a "locking" signal. All spectra were recorded at least five times at a sweep width of 50 Hz under conditions of slow passage and negligible saturation. Nuclear magnetic resonance of $^7Li^+$ was achieved using the same spectrometer system at 19 MHz in a "non-locking" field-swept mode. The magnetic field strength was adjusted in each experiment until resonance was obtained.

Spin–lattice relaxation times (T_1) of the lithium ion nucleus were determined by the method of adiabatic rapid passage,[12,13] which depends on the reversal of the magnetization vector by a large radiofrequency (H_1) field. This was accomplished experimentally by saturating the lithium ion nmr absorption signal, and then recording the dispersion (in-phase) signal concomitant with asymmetrical triangular modulation of the main (H_0) magnetic field with a Hewlett-Packard HP 3300-A function generator. The modulation sweep period was adjusted until the amplitude of the return trace had vanished. Sweep periods were measured by means of an electronic counter (Hewlett-Packard HP 52564). The entire nmr relaxation experiment was photographed from an oscilloscope pattern on Polaroid film (ASA 3000). Measurements were made directly on these photographs and T_1 was calculated as described below. A precision in T_1 of ±3% was attained by this procedure.

(6) A. Berger, A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, **81**, 62 (1959); M. Takeda and E. O. Stejskal, *ibid.*, **82**, 25 (1960); J. E. Bundschuh and N. C. Li, *J. Phys. Chem.*, **72**, 1001 (1968).

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(8) S. O. Nielsen, *Biochim. Biophys. Acta*, **37**, 146 (1960); I. M. Klotz and B. H. Frank, *Science*, **138**, 830 (1962); I. M. Klotz and B. H. Frank, *J. Amer. Chem. Soc.*, **87**, 2721 (1965); see also ref 6.

(9) R. G. Bates in "Determination of pH," John Wiley and Sons, Inc., New York, N. Y., 1964.

(10) D. Rosenthal and J. S. Dwyer, *Anal. Chem.*, **35**, 161 (1963).

(11) Y. Nozaki and C. Tanford, *J. Amer. Chem. Soc.*, **89**, 736 (1967).

(12) L. E. Drain, *Proc. Phys. Soc.*, **62A**, 301 (1949).

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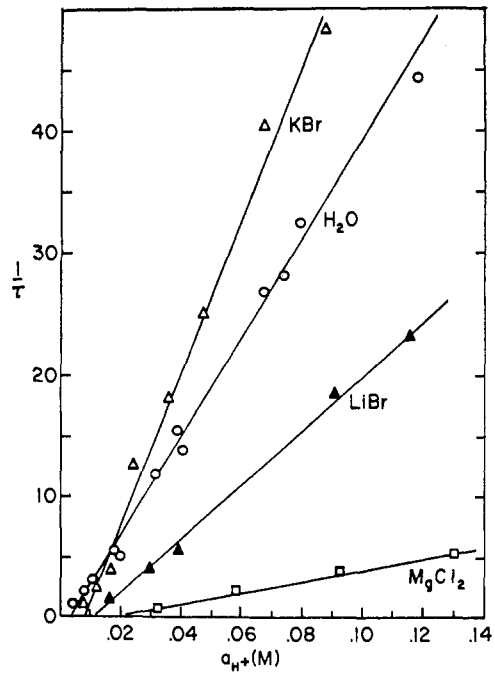


Figure 1. Representative proton exchange rate data for N-methylacetamide in aqueous solution plotted as τ^{-1} *vs.* hydrogen ion activity. The slope of the plot gives the second-order rate constant (k_{H^+} in M^{-1} sec^{-1}) and the intercept k_{H_2O} (in sec^{-1}). Additional points were measured at $a_{H^+} > 0.14$, but are omitted from this figure.

Analysis of Nmr Line Shapes. The line shapes of the nmr signals were analyzed according to published procedures.[6] Under exchange conditions resulting in a doublet, the methodology of Berger, *et al.*,[6,14] was used, while for a broadened singlet the procedure of Takeda and Stejskal was employed.[6,14,15] The best value of τ (the mean time between exchanges) for each line shape was obtained by means of a computer program written in Basic for a G.E. 265 computer which varied τ systematically until the best match of the calculated with the experimental line-shape parameters was attained.

Analysis of Kinetic Data. Assuming that hydroxide ion catalysis of exchange is negligible on the acid side (pH 0.5–3.0) of the exchange minimum, and also that direct amide–amide exchange does not occur,[6] eq 2 may be written for τ and the relevant kinetic constants

$$\frac{1}{\tau} = \frac{\text{rate}}{a_A} = k_{H^+}a_{H^+} + k_{H_2O} \qquad (2)$$

where a_A refers to the thermodynamic activity of amide in the exchange system, k_{H^+} is the acid-catalyzed second-order rate constant for proton exchange in units of M^{-1} sec^{-1}, k_{H_2O} is the pseudo-first-order rate constant for water-catalyzed proton exchange in units of sec^{-1}, and a_{H^+} is the activity of the hydronium ion. Thus the slope of a plot of τ^{-1} (defined as the specific rate, R) *vs.* a_{H^+} (as measured by the glass electrode) is equal to k_{H^+}, while the intercept equals k_{H_2O}. All fitting of data in the determination of rate parameters was accomplished by standard least-squares methods. Uncertainty values are the standard deviations calculated from the least-squares constants and the experimental points.

Calculation of the Spin–Lattice Relaxation Times. This determination was most conveniently carried out by adjusting the asymmetric modulation sweep period until the amplitude of the dispersion signal on the return trace had vanished. Under these conditions the spin–lattice relaxation time (T_1) is evaluated from eq 3,[11,12] where t_1 and t_2 are sweep periods in seconds for the forward and return traces. Equation 3 is most easily solved for T_1 by an iterative computer procedure which involves varying T_1 until the left- and right-hand sides of the equation are equal.

$$e^{-t_2/T_1} = 0.5(1 + e^{-(t_1+t_2)/T_1}) \qquad (3)$$

Results

Berger, *et al.*,[6] have demonstrated that the rate of exchange of the amide hydrogen of NMA can be determined by measurements of the splitting of the N-methyl proton resonance which is induced by spin–spin interaction with the amide hydrogen. As the exchange rate increases, the initially sharp N-methyl doublet broadens and then coalesces into a singlet. The values obtained by such measurements for the acid-catalyzed second-order rate constant (k_{H^+}) for proton exchange, intercept values, relative viscosities, and relative (to water) rate constants (k/k_0) for N-methylacetamide in water and 19 aqueous electrolyte solutions are listed in Table I. The value of k_{H^+} (in water at 23°) of 435 ± 20 M^{-1} sec^{-1} determined in this study is in good agreement with that obtained by Berger, *et al.*[6] (380 ± 40 M^{-1} sec^{-1} at 23°), and by Bundschuh and Li[6] (398 M^{-1} sec^{-1} at 25°) but in rather poor agreement with the value reported by Takeda and Stejskal[6] (200 ± 70 M^{-1} sec^{-1} at 25°). Figure 1 shows some of the actual specific rate data plotted against hydronium ion activity.

The results presented in Table I and Figure 1 show that high concentrations of neutral salts do affect the rate of exchange of the amide hydrogen in NMA and that variations in the nature of the supporting cation have a much larger effect than changes in the supporting anion (except SCN^-). Thus we may note in Table I that k/k_0 (where k is the second-order rate constant for acid-catalyzed exchange in the salt solution (k_{H^+} and k_0 for exchange under the same conditions in pure water) is closely similar for all the sodium salts tested, and also quite constant (at different values in each case) for the Li^+ and K^+ series. Further, some cations (*e.g.*, K^+, Rb^+, and TMA^+) markedly increase the exchange rate of the NMA amide proton relative to the rate in water, while other cations (*e.g.*, Li^+ and Mg^{2+}) strikingly decrease the relative rate. The relatively bulky tetraethylammonium (TEA^+) cation, however, exerts little or no effect on the rate of acid-catalyzed proton exchange.

(14) A spin–spin coupling constant of 5.19 Hz was used for nonexchanging conditions (see ref 6). Extrapolation of the N-methyl doublet separation to pH 5 (the pH of minimum exchange) gives an apparent splitting of 5.20 Hz. Correction for a spin–spin coupling constant of about 0.5 Hz between C-methyl and N-methyl protons was neglected in this study.

(15) A typographical error exists in the original publication. Takeda and Stejskal's eq 6 should read

$$\frac{\delta\omega_{1/2}}{\delta\omega} = \left\{\left[\left(\frac{1}{t}+\frac{1}{Q}\right)^2\left(\frac{2}{\frac{2}{t}+\frac{1}{Q}}-\frac{8}{t}\right)^2 + \left(\frac{4}{Q}\left[\frac{2}{t}+\frac{1}{Q}\right]+1\right)^2\right]^{1/2} - \left(\frac{1}{t}+\frac{1}{Q}\right)\left(\frac{2}{\frac{2}{t}+\frac{1}{Q}}-\frac{8}{t}\right)\right\}^{1/2}$$

Table I. Kinetic Parameters for Acid-Catalyzed Proton Exchange in N-Methylacetamide[a]

Salt	k_{H^+}, M^{-1} sec^{-1}	Intercept value	No. of pts	k/k_0[b]	η/η_0[b]
Water	435 ± 20	−2.67 ± 1.32	17	1.00	1.00
NaCl	360 ± 11	−2.47 ± 0.87	10	0.83 ± 0.05	1.26
NaBr	368 ± 29	−4.87 ± 1.90	7	0.85 ± 0.08	1.03
NaI	343 ± 21	−3.39 ± 1.46	11	0.79 ± 0.06	0.89
LiCl (1.5 *M*)	282 ± 14	−2.58 ± 0.93	8	0.65 ± 0.04	1.16
LiCl (2.8 *M*)	175 ± 22	−4.03 ± 1.88	8	0.40 ± 0.05	1.79
LiCl (4.5 *M*)	110 ± 9	−2.08 ± 0.70	4	0.25 ± 0.02	1.65
LiBr	253 ± 12	−4.33 ± 1.96	9	0.58 ± 0.04	1.19
LiI	198 ± 22	−3.94 ± 2.70	8	0.46 ± 0.06	1.00
KCl	559 ± 14	+0.70 ± 1.35	6	1.29 ± 0.07	0.92
KBr	622 ± 21	−4.20 ± 0.95	8	1.43 ± 0.08	0.79
KI	529 ± 24	+0.42 ± 1.42	5	1.22 ± 0.08	0.69
RbCl	624 ± 20	+0.85 ± 0.82	6	1.44 ± 0.08	0.80
NaSCN	523 ± 5	−2.79 ± 0.25	5	1.22 ± 0.06	1.14
$MgCl_2$	69 ± 3	−2.33 ± 0.52	5	0.16 ± 0.01	3.22
$NaClO_4$	345 ± 11	−7.41 ± 1.82	6	0.79 ± 0.04	1.03
TMACl	537 ± 36	−0.06 ± 1.62	8	1.24 ± 0.10	1.95
TMABr	660 ± 25	−4.97 ± 1.44	8	1.52 ± 0.09	1.62
TEACl	450 ± 13	−1.06 ± 0.64	7	1.04 ± 0.06	5.06
TEABr	396 ± 15	0 ± 1.01	9	0.91 ± 0.05	4.83

[a] All ionic solute concentrations are 2.8 *M* unless otherwise noted; temperature = 23 ± 1°. [b] Relative to 1 *M* NMA in water.

Figure 1 and Table I also show that the intercept in the plots R (τ^{-1}) *vs.* a_{H^+} are all very close to zero, indicating that k_{H_2O} is very small, in agreement with the conclusions of earlier exchange studies in water.[6] The fact that the intercept is consistently slightly (beyond experimental error) negative probably is due to a small systematic error in the line-shape analyses which results from the "semiclassical" analytic treatment used.[16]

The dependence of the rate constant on salt concentration was determined for LiCl. The results are depicted in Figure 2, and show that for this salt (and we assume for the other salts as well) log k/k_0 is a linear function of salt concentration. It should be emphasized that viscosity differences of the various solutions are explicitly taken into account in the line-shape analysis as an effective line width for the nonexchanging C-methyl protons of the NMA molecule.

The spin–lattice relaxation times of the lithium ion nucleus were determined under conditions of constant lithium salt concentration and varying mole fraction of amide, with the total solvent mole fraction held constant, in an effort to obtain further insight into the details of the cation amide interaction. These data for the various lithium halides, together with the relevant viscosity data, appear in Table II and also in graphical form in Figure 3. At the concentrations studied (total moles of solvent equals 110 times the moles of lithium halide) the reciprocal of the viscosity-corrected relaxation time $(T_1\eta_{rel})^{-1}$ varies continuously with increasing mole fraction of amide. Furthermore, this variation, within experimental error, is the same for all of the halide salts, suggesting (as discussed below) that the interaction of the amide dipole with the lithium ion is not strongly affected by the nature of the associated anion and varies continuously with changes in the bulk composition of the solvent.

Table II. Lithium Ion Nucleus T_1 Relaxation Times[a] in Aqueous N-Methylacetamide Ionic Solutions (Temperature = 23 ± 1°)

Salt	NMA mole fraction	T_1,[b] sec	η_{rel}[c]	$T_1\eta_{rel}$
LiCl	0.091	7.12	2.74	19.51
LiCl	0.242	2.91	5.88	17.11
LiCl	0.343	2.14	7.02	15.02
LiCl	0.495[d]			
LiBr	0.091	7.92	2.39	18.93
LiBr	0.242	2.97	5.57	16.54
LiBr	0.343	2.00	7.31	14.62
LiBr	0.495	1.55	7.96	12.34
LiI	0.091	8.69	2.12	18.42
LiI	0.242	3.53	4.84	17.01
LiI	0.343	2.49	6.25	15.56
LiI	0.495	1.63	7.64	12.45

[a] Conditions: 0.01 mol of lithium halide, 1.10 mol of solvent (water + amide). [b] Out-gassing with helium did not alter relaxation times. [c] Relative to pure water. [d] LiCl is not completely soluble at this mole fraction of NMA.

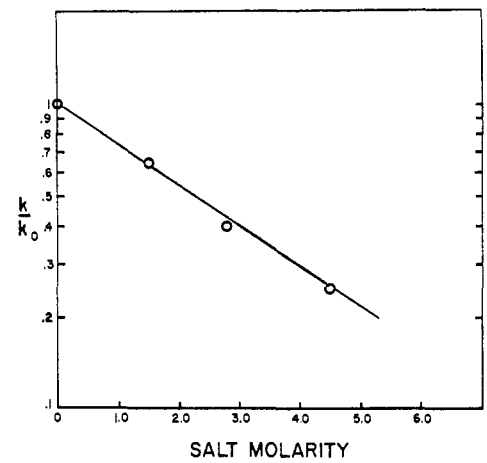


Figure 2. Relative (to water) proton exchange rate constants for N-methylacetamide as a function of lithium chloride concentration; temperature = 23 ± 1°.

Discussion

In this study, we have observed that added electrolytes can markedly increase, decrease, or not change the

(16) The question of systematic errors inherent in various nmr line-shape analyses has been analyzed in detail by A. Allerhand, H. S. Gutowsky, J. Jonas, and R. A. Meinzer, *J. Amer. Chem. Soc.*, 88, 3185 (1966).

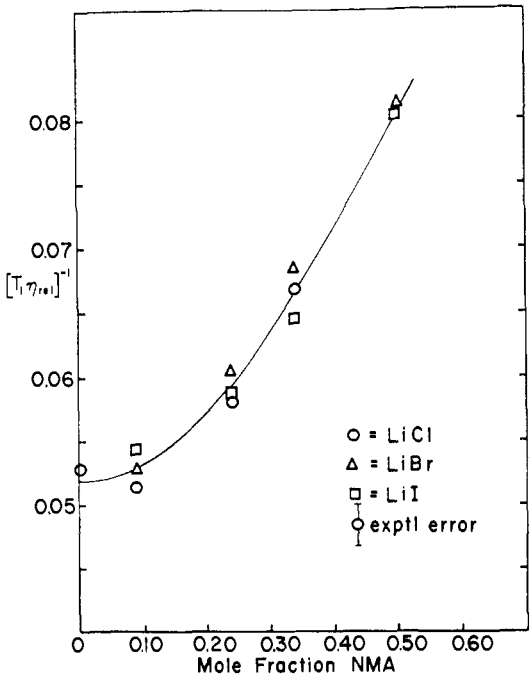


Figure 3. Spin-lattice relaxation time (T_1) for $^7Li^+$ in NMA–water solutions as a function of amide mole fraction.

second-order rate constant for the acid-catalyzed proton exchange of NMA. Furthermore, it has been shown that the direction and magnitude of this effect depend primarily on the nature of the supporting cation. Before attempting mechanistic interpretations of these results, it is important to consider whether they might be attributable to experimental artifact.

The chief potential source of such artifacts is erroneous pH measurement in these concentrated ionic solutions. Several studies, however, have shown that the potential read by the glass–calomel combination electrode reflects changes in hydronium ion activity with only a small error arising from liquid junction potentials.[9-11] These liquid junction errors have been estimated in the present study to be less than 0.04 pH unit at 25°.

Noyes[17] has shown that reaction rates are independent of solvent structure and viscosity effects for chemical reactions with second-order rate constants which are much less than $10^7\ M^{-1}\ sec^{-1}$. Since the rate constants for acid-catalyzed proton exchange in NMA are of the order of several hundred, these factors, which are important in determining the rate of diffusion-controlled reactions, are not of concern here. As has been pointed out in the Results, viscosity effects on the nmr spectral line shapes are automatically taken into account by the inclusion of an effective T_2 (spin–spin relaxation time) for a nonexchanging proton. The C-methyl protons of NMA were used for this purpose here, as well as in other studies.[6]

Salt-induced changes in H_0, the Hammett acidity function which is a measure of the propensity of a solvent to donate a proton to an uncharged molecule, seem unlikely to be an explanation of the observed salt effects in view of the data of Rosenthal and Dwyer[10] which show that for variously concentrated lithium chloride solutions the change in pH is an accurate reflection of changes in H_0 (H_0 − pH = −0.10 over a LiCl concentration range of 1–4 M for the base, *p*-nitroaniline). Comparison of (H_0 − pH) values for other salt solutions and bases lead to similar conclusions.

The results presented in this paper and elsewhere[6] have shown that the observed specific rate of exchange of amide protons in acid solution can be satisfactorily represented by eq 2. Furthermore, it has been shown that the water catalysis term is negligibly small, and (by Berger, *et al.*[6]) that the specific rate of exchange is independent of amide concentration. This latter observation demonstrates that direct amide–amide exchange does not contribute appreciably to the over-all rate, and that the observed specific rate is independent, as expected, of the activity coefficient of the amide (to the extent that the activity coefficient of NMA varies with amide concentration between 0 and 5 M). How then do the various neutral salts affect the observed specific exchange rate? Again it seems fairly clear that it is not through an effect on the activity coefficient of the amide, since Schrier and Schrier[18] have measured the activity coefficient of NMA in various aqueous electrolytes directly by distribution techniques and have shown that *both* cations and anions have profound effects on the activity (*i.e.*, the effects on the activity coefficients do not correlate at all with those here observed on the kinetics of exchange).

The rate constant (k_{H^+}) for the nonideal acid-catalyzed exchange reaction may be expressed in terms of transition-state theory as

$$k_{H^+} = \frac{1}{\tau a_{H^+}} = \frac{\text{rate}}{a_A a_{H^+}} = \frac{kT}{h}\left(\frac{K^{\pm}}{\gamma^{\pm}}\right) \qquad (4)$$

where τ is the mean time between exchanges (see eq 2), a_{H^+} is the activity of the hydronium ion, a_A is the activity of amide, k is Boltzmann's constant, h is Planck's constant, T is the absolute temperature, $K^{\pm}$ is the "pseudo" equilibrium constant defining the formation of the transition-state complex, and $\gamma^{\pm}$ is the activity coefficient of the transition-state complex.[19]

Thermodynamically, we may attribute the observed salt ion effects on k_{H^+} to changes in $\gamma^{\pm}$ with electrolyte type and concentration. Thus the *relative* rate constant is given by

$$k/k_0 = \gamma_0^{\pm}/\gamma^{\pm} \qquad (5)$$

where the subscript zero denotes the salt free or amide–water system. This system will be taken as our standard state, and hence $\gamma_0^{\pm} = 1$.

We expect ion–dipole interactions to be involved in the effect of salts on the exchange process, and since it is well known that ion–dipole interactions typically show a direct dependence of the activity coefficient of the nonelectrolyte on ionic strength (or salt concentration for uni-univalent electrolytes),[20] we may write

$$\log (k/k_0) = \alpha[\text{salt}] \qquad (6)$$

where α is simply a proportionality constant. Figure 2 shows that the dependence of the exchange rate of NMA

(17) R. M. Noyes, *Progr. Reaction Kinetics*, **1**, 129 (1961).

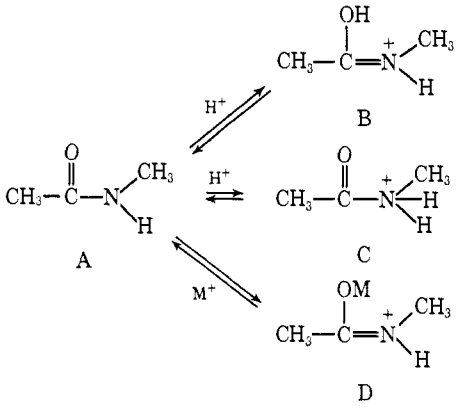
(18) E. E. Schrier and E. B. Schrier, *J. Phys. Chem.*, **71**, 1851 (1967).

(19) For a detailed discussion the reader is referred to I. Amdur and G. G. Hammes in "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1966.

(20) J. W. Belton, *Trans. Faraday Soc.*, **33**, 653 (1937); see also a discussion of the Kirkwood ion–dipole electrostatic interaction theory in J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958, Chapter 5.

on LiCl concentration has exactly the form predicted by eq 6.

Thus we have attributed the observed dependence of the exchange rate on salt type to effects on $\gamma^{\pm}$, but this provides little insight into mechanism. A possible mechanistic proposal may be developed out of the suggestion of Berger, *et al.*,[6] that NMA can exist in acid solution as two protonated species (B and C) in equilibrium with the unchanged form (A).

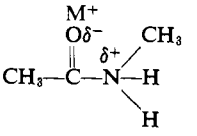


These workers have attributed the unusually slow acid-catalyzed exchange of the amide hydrogen of NMA in acid solution to an equilibrium which greatly favors the non-amide hydrogen exchanging species (B) over species C which is kinetically active in the exchange of the amide hydrogen.

Ions can interact with this system in a number of ways. In general, we would expect anions to associate with protonated NMA in the vicinity of the nitrogen. Cations would probably be attracted to the oxygen, but could associate with it in at least two distinct fashons: (a) cations of high charge density could form ion-pair complexes with NMA (species D in eq 8); (b) all cations can form ion–dipole complexes with unprotonated carbonyl oxygens (species C).

We have shown (Table I) that k/k_0 increases progressively in the following order for the various cations: $Mg^{2+} < Li^+ < Na^+ < TMA^+ \simeq K^+ < Rb^+$. Thus k/k_0 seems to increase with decreasing cationic charge density. This trend is examined more quantitatively in Figure 4, where we plot log k/k_0 against the reciprocal of the cationic crystal radii for the univalent alkali cations.[21] Clearly over this range of ion size the points approximate a straight line (with log k/k_0 changing sign from minus to plus between Na^+ and K^+), and thus for these ions log k/k_0 is also directly proportional (through the Born equation) to the free energy of cationic hydration. (Obviously, this linear relation breaks down in the limit of very large ions, where k/k_0 again approaches unity; see TEA^+, Table I.)

These results can be rationalized in terms of the above model by speculating that the cations of high charge density can form stable ion-pair complexes with NMA (species D). This results in shifting the equilibria of eq 8 so as to reduce the fraction of NMA molecules in the catalytically active form (C). All the ions will tend to form ion–dipole complexes with the unprotonated carbonyl oxygens of NMA, thereby apparently stabilizing the transition-state complex. As the competitive formation of species D is decreased for the low charge density cations, structures such as



will tend to form in increasing amounts and stabilize the N-protonated species and thus enhance the amide hydrogen exchange. Very large, low charge density cations such as TEA^+ would be quite ineffective in forming any type of stable complex with NMA, and thus should have little effect on the exchange rate in accord with experimental findings (Table I).

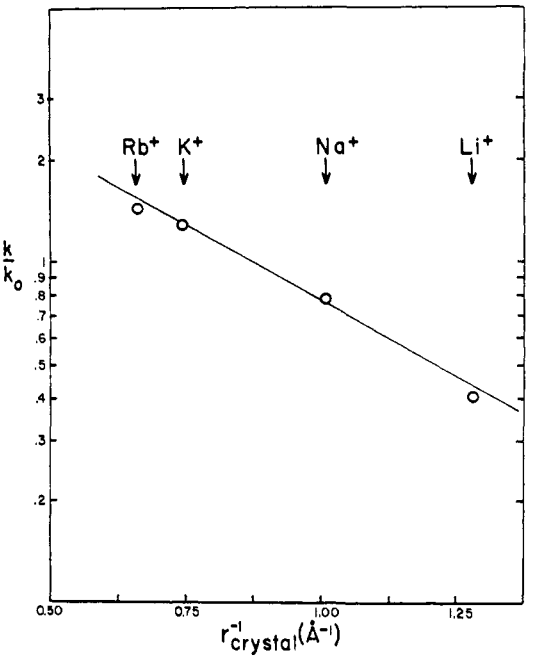


Figure 4. Relative proton exchange rate constants (for chloride salts) *vs.* the reciprocal of cationic crystal radii for N-methylacetamide (for details see text); temperature = 23 ± 1°; ionic radii from ref 21.

Models of this kind are supported by X-ray diffraction studies on crystals of salt–amide complexes[22] which generally locate the cation adjacent to the carbonyl oxygen of the amide, while the anion is bound in the vicinity of the amide nitrogen. This picture is also consistent with the view that most of the spherical anions are sufficiently far removed from the carbonyl oxygen to have little or no effect on the cation binding to the locus. However, for highly dipolar anions such as SCN^-, the interaction between amide and anion might be partially of the dipole–dipole type, and the positive end of the SCN^- dipole might come close to the carbonyl oxygen and thus perturb the cation binding interaction somewhat.

The above model, attributing the cation dependence of exchange on shifts in the equilibrium between protonated species of NMA, can be tested directly. We would expect that (1) base-catalyzed exchange, which proceeds through a single deprotonated intermediate, should not show the same cation dependence; and (2)

(21) Data of V. M. Goldschmidt cited in Table 42 of R. W. Gurney in "Ionic Processes in Solution," McGraw-Hill Book Co., Inc., New York, N. Y., 1953.

(22) J. Bello and H. R. Bello, *Nature*, **190**, 440 (1961); J. Bello and H. R. Bello, *ibid.*, **194**, 681 (1961); D. J. Haas, *ibid.*, **201**, 64 (1964); J. Bello, D. J. Haas, and H. R. Bello, *Biochemistry*, **5**, 2539 (1966).

that increasing the strengths of the electrostatic interactions between amide and cation in the acid-catalyzed exchange reaction (*e.g.*, by conducting the study in 50% aqueous dioxane) should increase the ease of ion-pair formation with the lower charge density cations and thus shift most of the cations into the class of exchange-rate reducers. These expectations are currently being subjected to experimental test, and the results will be reported elsewhere.

The above model also suggests that cations and anions are well separated in the salt–amide complex, and that the cationic environment might be quite insensitive to the nature of the anion located near the amide nitrogen. This prediction can be examined directly by the method of ion nucleus spin–lattice relaxation measurements. This approach is particularly sensitive because of the existence of nuclear quadrupole moments, which are associated with nuclear spin quantum numbers in excess of one-half. Quadrupole moments, coupled with fluctuating electric field gradients, dominate nuclear spin–lattice relaxation times, overshadowing contributions from magnetic dipolar interactions. The important factors in establishing a nonvanishing electric gradient are the details of symmetry and distance of the nuclear environment, while fluctuations, with particular correlation times, in these symmetry and distance terms are responsible for establishing the *characteristic* relaxation rate. Gross changes in symmetry, such as the substitution of one molecule for another in the vicinity of the resonating nucleus, will change both static and dynamic aspects of the electric field gradient, and hence nuclear relaxation rates.[13]

Studies by Craig and Richards[23] of lithium ion nucleus spin–lattice relaxation rates in aqueous solution as a function of lithium chloride concentration have revealed that the primary contribution to the electric field gradient arises from the dipole moments of the solvent (water) molecules. In other words, ionic contributions to the relaxation rate are negligible. Thus in binary solvent systems of high dielectric constant, the viscosity-corrected nuclear spin–lattice relaxation rate should become a measure of differential ion–solvent interaction, provided that the two components differ in the magnitude of their dipole moments.

The following naive physical picture can be constructed for the nuclear relaxation rate behavior of an ion in a mixed binary solvent system, *e.g.*, water and an amide. In pure water the ion is surrounded by a hydration sphere of water molecules; fluctuations in spherical symmetry and distance by thermal motion result in a characteristic contribution to the relaxation rate. Now we imagine the water molecules being replaced one by one by amide molecules, resulting in electric field gradient changes, and thus in changes in the nuclear relaxation rate, since the amide dipole moment is about 3 D, and that of the water molecule is only about 1.8 D. When this process has gone to completion the ionic hydration sphere is fully replaced by amide molecules, which now impart a characteristic new "terminal" relaxation rate. Thus by keeping the amount of lithium ion (in this case) constant and varying the mole fraction of amide in the amide–water solvent, information about the relative interaction of amide and water with $^7Li^+$ can be obtained.

In the light of this picture, it seems reasonable to use $1/(T_1\eta_{rel})$ as a probe of the lithium ion–amide interaction. The experiments reported in this paper show that the variation in $1/(T_1\eta_{rel})$ (at constant lithium ion mole fraction) with increasing mole fractions of amide (at constant *total* moles of solvent) is the same, within experimental error, regardless of the particular halide anion present. This finding is consistent with the model of the amide–ion interaction presented above. Further studies with other cationic nuclei are currently in progress.

This paper reports on one of a number of experimental approaches designed to probe the details of the interactions of ions and amides in solution. It is hoped that the application of a variety of such experimental probes will eventually serve to "map" the molecular details of the interaction between the amide group and various specific ions. Meanwhile, however, on a more pragmatic level, the finding that electrolytes can induce specific changes in the rate of proton exchange of a simple unstructured amide has direct implications for the use of hydrogen exchange in the study of macromolecules in concentrated electrolyte solutions.

Acknowledgment. Most of the experimental work reported in this paper was carried out while the authors were members of the Biochemistry Department, Dartmouth Medical School, Hanover, N. H. We are very grateful to Professors Paul R. Shafer and Karl Kuhlmann of the Dartmouth Chemistry Department for making their nmr equipment (supported by a grant from the National Science Foundation) available to us, and for providing much technical help and advice. We are also pleased to acknowledge the participation of Mr. Michael Evans, Mr. David Watts, and Mrs. Virginia Schleich in various phases of this work. This research was supported by Research Grants AM-03412 and AM-12215 and Research Career Program Award GM-K3-5479 (to P. H. v. H.) from the U. S. Public Health Service, and a Helen Hay Whitney Foundation postdoctoral fellowship (to T. S.).

(23) R. A. Craig and R. E. Richards, *Trans. Faraday Soc.*, **59**, 1972 (1963).