Proton Exchange of *N*-Methylacetamide in Concentrated Aqueous Electrolyte Solutions. II. Acid Catalysis in Water–Dioxane Mixtures and Base 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 containing 20% (v/v) dioxane. As found previously in the absence of dioxane (T. Schleich, R. Gentzler, and P. H. von Hippel, *J. Amer. Chem. Soc.*, **90**, 5954 (1968)) second-order rate constants for the exchange process (k), determined by nmr line-shape analyses, are markedly dependent on the nature of the cation present and quite insensitive to the species of supporting anion. Also it is shown that log k is a linear function of electrolyte concentration. The lowering of the solvent dielectric constant which results from the addition of dioxane to the system augments the exchange rate reducing effects of the high charge density cations (*e.g.*, Li^+). This decrease in solvent dielectric constant also reduces the effectiveness of the lower charge density ions (*e.g.*, K^+, Rb^+) in increasing the exchange rate of the amide hydrogen. These results can be interpreted in terms of the previously proposed model of a cation-dependent alteration in the equilibrium between various tautomeric forms of protonated NMA, not all of which represent catalytically active species in the acid-catalyzed proton exchange reaction. The base-catalyzed proton exchange kinetics of NMA have also been examined. Both cations and anions show specific effects on the kinetics of this process, but these results appear to be explicable in terms of simple complexing of the ions with the amide dipole.

The kinetics of exchange with solvent of peptide hydrogens in proteins are often used by physical biochemists as measures of the degree of exposure of these groups to the aqueous environment, *i.e.*, as a conformational probe. Recent work has demonstrated that concentrated electrolyte solutions can usefully be employed to alter the conformational stability of proteins and nucleic acids (for recent reviews see ref 1), and thus hydrogen exchange studies may often be conducted in the presence of these "denaturants" in the future. To interpret changes in macromolecular hydrogen exchange rates in the presence of denaturants, one must know whether these changes are to be attributed to effects on the conformation of the macromolecules, or to effects of the solvent additive directly on the intrinsic "chemical" exchange rate of the fully exposed amide hydrogen. In order to obtain such information, as well as to examine further the mechanisms of ion–amide interactions, we initiated a study of the acid-catalyzed proton exchange kinetics of the amide proton of *N*-methylacetamide (NMA) in aqueous electrolyte solutions.

Our original investigation of this system[2] revealed a marked dependence of the second-order rate constant for the exchange process on the type of electrolyte used. It was found that the nature of the cation present was predominantly responsible for establishing the altered exchange rates. A change of anion (in the presence of a common cation) affected the rates very little. Cations of high charge density (*e.g.*, Li^+ and Mg^{2+}) were shown to be quite effective in reducing the acid-catalyzed rate constant, while cations of lower charge density (*e.g.*, K^+ and Rb^+) *increased* the magnitude of the rate constant relative to that observed for the amide–water system alone. These results were interpreted by invoking specific interactions between the ions and the amide dipole, leading to an altered equilibrium distribution of amide resonance (tautomeric) structures.

In view of the marked dependence of ion–dipole interactions on the dielectric constant of the surrounding environment,[3] it seemed that certain predictions of the above model could be tested by examining the effect on the exchange rates of a decrease in solvent dielectric constant (D). In addition, the tautomeric forms present under acid conditions would not be expected to perturb the exchange process at alkaline pH and so the effects of neutral salts on the kinetics of the "simple" base-catalyzed exchange process should also be revealing. To this end we have carried out, and report here, a series of measurements of the acid-catalyzed exchange of the amide proton in aqueous dioxane electrolyte solutions. Some preliminary data on the effects of neutral salts on the base-catalyzed exchange process are also described.

Experimental Section

Chemicals. All inorganic chemicals employed in this study were of reagent quality. The *N*-methylacetamide was Eastman White Label grade and was used as received, since previous analyses[2] indicated only a trace of impurity. Dioxane was reagent grade and it was further purified (to remove peroxides) by passage through a column of alumina.[4] It was then stored under nitrogen in an amber bottle until used. Hexamethyldisiloxane (HMDS) was purchased from K & K Laboratories.

Preparation of Solutions. All solutions used in the acid-catalyzed studies were prepared in glass-distilled water containing 20% (v/v) purified *p*-dioxane (22.6% w/w *p*-dioxane) 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 removed and placed

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(1) For background references and discussion of this topic see P. H. von Hippel and T. Schleich, *Accounts Chem. Res.*, 2, 257 (1969).

(2) T. Schleich, R. Gentzler, and P. H. von Hippel, *J. Amer. Chem. Soc.*, 90, 5954 (1968).

(3) For a detailed discussion of ion–dipole interaction theory see: J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958, Chapter 5.

(4) W. Dasler and C. D. Bauer, *Ind. Eng. Chem., Anal. Ed.*, 18, 52 (1946).

in an nmr tube and immediately sealed with a pressure cap (Wilmad). The solutions used in the base-catalyzed studies were prepared in the same manner as previously described (minus dioxane) except that 0.001 *M* veronal was added to serve as a stabilizer of the hydrogen ion concentration. Adjustment to the desired pH was accomplished with dilute NaOH (CO_2 free). All solutions used in the rate studies were run on the nmr spectrometer within 6-8 hr after preparation.

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 solutions. Titrations to the desired pH were carried out at 35 ± 1° in a jacketed reaction vessel. The electrode was standardized against pH 1.00 and 4.00 Sørensen buffer (Brinkman) and small corrections to the observed pH, when necessary, were made by linear interpolation. Control experiments, using potassium hydrogen phthalate as a reference,[5] revealed essentially no pH changes (no greater than 0.01 pH unit) of these Sørensen buffers in going from 20 to 35°. Other control experiments revealed no major changes (less than 0.01 unit) in pH of the adjusted aqueous dioxane–amide–salt solutions over a time period of 6 hr.

The same titration apparatus, with the reaction cell protected by a nitrogen velocity barrier, was used to achieve the necessary alkaline pH in the base-catalyzed studies. A pH 7.00 Sørensen buffer was employed as a reference. No changes in pH could be observed in an adjusted sample containing 0.001 *M* veronal over a time interval of at least 6 hr.

Large liquid junction potentials, leading to appreciable errors in pH determination, are not expected for concentrated aqueous salt solutions.[6] In aqueous dioxane solutions liquid junction potentials have also been shown to be small,[7] and are usually neglected. Thus the observed (corrected) pH was set to the negative logarithm of the hydrogen ion activity.

Nmr Spectrometer Details. All proton spectra were recorded at 100 MHz using a Varian HA-100 spectrometer system at a probe temperature of 35 ± 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 in the frequency swept mode at a sweep width of 50 Hz under conditions of slow passage and negligible saturation.

Analysis of Nmr Line Shapes. The line shapes of the nmr signals were analyzed as previously described.[2,8] The best value of τ (the mean time between exchanges) for each line shape was obtained by means of a computer program written in Fortran IV for an IBM system 360/50 computer which varied τ systematically until the best match of the calculated with the experimental line-shape parameters was attained.

Analysis of Kinetic Data. The acid-catalyzed kinetic data were treated as previously described[2] using the following expression

$$\tau^{-1} \equiv \frac{\text{rate}}{a_A} = k_{H^+}a_{H^+} + k_{H_2O} \tag{1}$$

where a_A refers to the thermodynamic activity of amide in the exchange system, k_{H^+} is the acid-catalyzed second-order rate constant 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 second-order rate constant, k_{H^+}, is obtained from the slope of a plot of τ^{-1} *vs.* a_{H^+} (as measured by the glass electrode). Note that τ^{-1} is *defined* as rate over a_A, but, τ^{-1} is *measured* directly from the nmr spectrum. Thus a_A does not enter into the measurement directly. For a further discussion of the development and mechanistic significance of eq 1 see ref 2. All data fitting in the determination of rate parameters were performed by standard least-squares methods. Uncertainty values are the standard deviations calculated from the least-square constants and the experimental points.

Base-catalyzed kinetic data were treated in the same manner except that a_{OH^-} replaces a_{H^+} in eq 1. The hydroxide ion activity (for dioxane-free solutions) was calculated from the usual expression

$$a_{OH^-} = \frac{K_w}{a_{H^+}} \tag{2}$$

where K_w is the ionization constant of pure water at 35° (2.084×10^{-14}) and a_{H^+} is calculated from the observed (corrected) pH above.

The employment of the pure water ionization constant in a concentrated aqueous electrolyte solution merits comment. The ionization constant of water, K_w, is defined in thermodynamic terms

$$K_w = \frac{\gamma_{H^+}\gamma_{OH^-}}{a_{H_2O}} m_{H^+}m_{OH^-} \tag{3}$$

as shown in eq 3. With increasing ionic strength, $\gamma_{H^+}\gamma_{OH^-}/a_{H_2O}$, the activity coefficient function, decreases monotonically from the pure water value of unity, and at approximately 0.5 *M* begins to increase in the same fashion. The exact rates of decline and rise are dependent on the nature of the particular salt. The ionization product, $m_{H^+}m_{OH^-}$, on the other hand, displays an almost even and opposite trend, *i.e.*, first increasing and then decreasing to the pure water value.[9] What is important in the present context is that the product of these terms very closely approximates the pure water value over a wide range of electrolytes and concentrations, thus justifying the use of the pure water value for K_w.

Results

Acid Catalysis in Water–Dioxane Mixtures. Dioxane was chosen as a diluent to lower the dielectric constant of the solvent environment because it is completely miscible with water, has no exchangeable protons, and has a very low dielectric constant ($D_{25°} = 2.1$). Furthermore, though no organic diluent can be expected to be completely "inert," in that it simply lowers the dielectric constant without bringing about significant changes in the molecular properties and structure of the aqueous solvent, dioxane is probably the best choice available. For example, the dielectric constant of dioxane–water mixtures is a linear function of composition (w/w) from 100 to 40% water, and the function $1/D(\mathrm{d}D/\mathrm{d}T)$ is approximately the same for water–dioxane mixtures as it is for water alone.[10] $D_{25°}$ for 20% dioxane–water (v/v) is 58.3.

The presence of dioxane in the aqueous amide electrolyte system does not abolish the previously observed pattern of neutral salt effects on the acid-catalyzed proton exchange of the amide hydrogen in NMA. Some typical results obtained in 20% aqueous dioxane are presented in Figure 1 and Table I. The data summarized in Table I show that electrolytes at high concentrations alter the acid-catalyzed rate constants of NMA in the dioxane–water system in much the same way as observed previously in the absence of dioxane.[2] Thus, as before, the ratio k/k_0 (k is the rate constant in the presence of salt and k_0 is the rate constant in the solvent without salt) varies considerably with changes in cation type (in the presence of a common anion), while this ratio is almost unaffected (within experimental error) by changes in anion type. Furthermore, as Table I shows, cations of high charge density (*e.g.*, Li^+) depress the rate of acid-catalyzed exchange, while those of low charge density (*e.g.*, K^+ and Rb^+) elevate the rate relative to the control (k_0).

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[5] R. G. Bates, "Determination of pH," Wiley, New York, N. Y., 1964, p 76.

[6] Reference 5, p 45; D. Rosenthal and J. S. Dwyer, *Anal. Chem.*, **35**, 161 (1963); Y. Nozaki and C. Tanford, *J. Amer. Chem. Soc.*, **89**, 736 (1967).

[7] I. M. Klotz and B. H. Frank, *ibid.*, **87**, 2721 (1965); ref 5, pp 223–229.

[8] A spin–spin coupling constant of 5.19 Hz was used for nonexchanging conditions. Correction for a spin–spin coupling constant of about 0.5 Hz between *C*-methyl and *N*-methyl protons was neglected in this study. Salt-induced changes in the magnitude of this coupling constant and the previous one for nonexchanging conditions were judged to be small, and hence neglected.

[9] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, p 641 (Figure 15-2-2).

[10] Reference 9, pp 161 and 717–718, provides detailed tabulations of the properties of dioxane–water mixtures.

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

Salt	k_{H^+}, M^{-1} sec^{-1}	Intercept value	No. of pts	k/k_0[b]
Water–dioxane	664 ± 11	−2.64 ± 1.00	7	1.00
LiCl (1.5 *M*)	332 ± 23	−4.77 ± 1.98	6	0.50 ± 0.04
LiCl (2.8 *M*)	180 ± 7	0.23 ± 1.02	7	0.27 ± 0.01
LiCl (4.5 *M*)	86 ± 5	−0.72 ± 0.86	7	0.13 ± 0.01
LiBr	226 ± 5	−2.16 ± 0.90	7	0.34 ± 0.01
LiI	173 ± 4	−0.28 ± 0.55	7	0.26 ± 0.01
NaCl	462 ± 7	−1.07 ± 0.67	7	0.70 ± 0.02
NaBr	476 ± 24	−2.47 ± 0.99	6	0.72 ± 0.04
NaI	406 ± 5	−1.65 ± 0.37	7	0.61 ± 0.01
KCl[c]				
KBr	832 ± 7	−2.11 ± 0.34	6	1.25 ± 0.02
KI	857 ± 14	−2.20 ± 0.97	7	1.29 ± 0.03
RbCl	1005 ± 22	−1.43 ± 0.50	8	1.52 ± 0.04

[a] All ionic solute concentrations are 2.78 *M* unless otherwise noted; solvent, 20% aqueous dioxane; temp, 35 ± 1°. [b] Relative to 1 *M* NMA in 20% aqueous dioxane. [c] This salt was not soluble to the extent of 2.78 *M* in the dioxane–water solvent.

Since our earlier study[2] was carried out at a temperature of 23°, and the present one at 35°, a selected set of salts was run at 35° in the absence of dioxane to correlate these findings with the earlier results. The data obtained are summarized in Table II. Comparison with Table I of ref 2 shows that k/k_0 for LiCl and RbCl is increased about 30% by this temperature change, while k/k_0 for NaCl is relatively unaffected. However, the significant point for the present comparison is that the relative rate constants (k/k_0) are markedly decreased in the presence of dioxane, relative to the value for the "water" control.

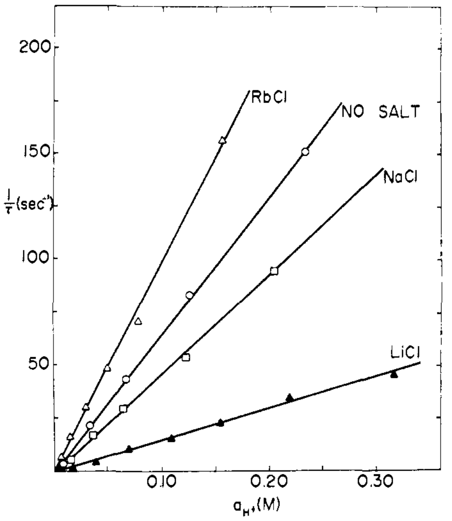


Figure 1. Representative proton exchange rate data for *N*-methylacetamide in aqueous dioxane electrolyte solutions plotted as τ *vs.* hydrogen ion activity. The slope of the plot gives the second-order rate constant (k_{H^+} in M^{-1} sec^{-1}) and the intercept k_{H_2O} (in sec^{-1}).

Table II. Comparison of Relative Rate Constants (Selected Salts) for Acid-Catalyzed Proton Exchange in Water and 20% Aqueous Dioxane at 35°

Salt	k/k_0[a] (H_2O)	k/k_0[a] (H_2O–dioxane)
LiCl	0.57 ± 0.02	0.27 ± 0.01
NaCl	0.82 ± 0.02	0.70 ± 0.02
RbCl	1.77 ± 0.04	1.52 ± 0.04

[a] Relative to 1 *M* NMA in solution minus electrolyte. Solute (ionic) concentrations are 2.78 *M*. k_0 (35°, H_2O) = 689 ± 12 M^{-1} sec^{-1}.

As observed in the previous study,[2] the intercept values taken from plots of specific rate (τ^{-1}) *vs.* a_{H^+} fall very close to zero, though consistently slightly below the origin. This probably represents a small systematic error in the line-shape analyses.

The dependence of k/k_0 on salt concentration was determined for LiCl. Figure 2 (and Table I) show that log k/k_0 decreases linearly with increasing salt concentration.

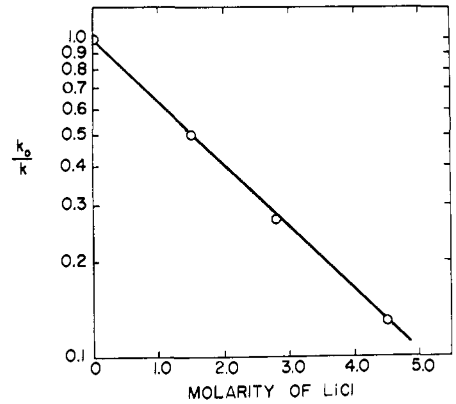


Figure 2. Relative (to water–dioxane) exchange rate constants for *N*-methylacetamide as a function of lithium chloride concentration; temp, 35 ± 1°.

Base Catalysis. We have also carried out a preliminary study of the effects of neutral salts on the base-catalyzed exchange of NMA in aqueous solution (in

Table III. Kinetic Parameters (k/k_0) for Base-Catalyzed Proton Exchange in *N*-Methylacetamide[a]

Salt	Li^+	Na^+	K^+	Rb^+
Cl^-	1.71	0.86	0.48	0.46
Br^-	1.52	0.67	0.40	
I^-	1.29	0.67		
ClO_4^-		0.40		

[a] All ionic solute concentrations are 2.78 *M*; NMA concentration is 1.0 *M*; pH stabilized with 0.001 *M* veronal buffer; k_0 = 3.5 × 10^6 M^{-1} sec^{-1}; temp, 35 ± 1°. A minimum of eight points was used in the determination of *k*. The maximum uncertainty in the k/k_0 values is ±0.05.

the absence of dioxane), using the same experimental techniques employed for the acid-catalyzed system. In this study it has been shown that *both* cations and anions are effective in altering the exchange rate, in contrast to the acid-catalyzed situation. Figure 3 shows some of our base-catalyzed data. Table III summarizes the findings to date and shows that: (1) Li^+ salts all increase k relative to k_0; (2) Na^+, K^+, and Rb^+ salts decrease k relative to k_0; and (3) for any common cation, k/k_0 decreases progressively with increasing anion size (polarizability) in the order $Cl^- > Br^- > I^- > ClO_4^-$.

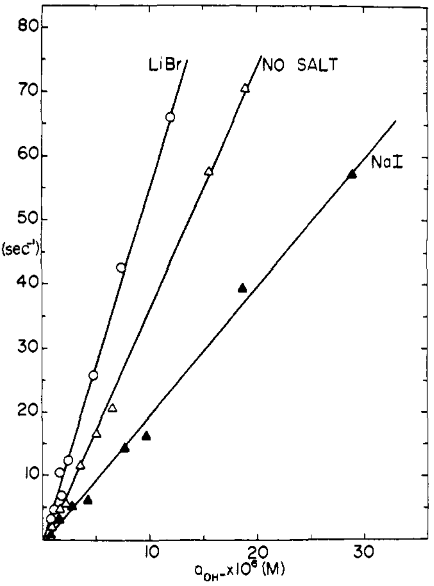


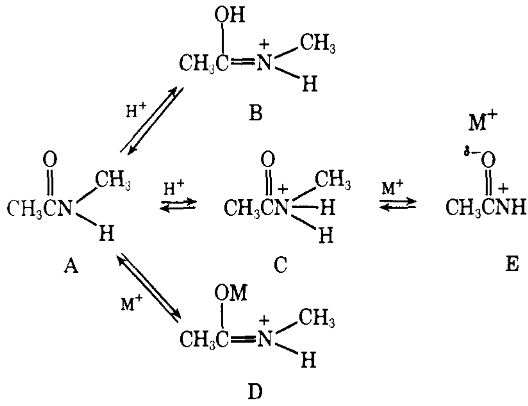
Figure 3. Representative proton exchange rate data for *N*-methylacetamide in aqueous (dioxane-free) electrolyte solutions plotted as τ^{-1} *vs.* hydroxide ion activity. The slope of the plot gives the second-order rate constant (k_{OH^-} in M^{-1} sec^{-1}) and the intercept k_{H_2O} (in sec^{-1}).

Discussion

Mechanisms for Acid Catalysis in Water–Dioxane Mixtures. This study clearly demonstrates that neutral salt effects on the acid-catalyzed proton exchange of the amide hydrogen in NMA in the presence of moderate amounts of dioxane are similar to those recorded in the absence of this component. The effect of dioxane, an additive which lowers the dielectric constant of the solvent milieu, is to *enhance further* the rate-depressing effect of high charge density cations (*e.g.*, Li^+), and to *decrease* the rate enhancing effect of low charge density cations (*e.g.*, Rb^+) relative to the dioxane-free system. As observed also in the absence of dioxane, the various anions exert almost no differential effects.

The modifications in neutral salt effects on the acid-catalyzed exchange of the amide proton of NMA brought about by the addition of dioxane to the solvent can be interpreted in terms of the mechanistic model proposed in our previous paper.[2] Thermodynamically, we had "explained" the observed results by invoking salt-induced changes in the activity coefficient of the transition-state complex leading to exchange, thus altering the rate of the reaction. In these terms one need simply add that a lowering of the solvent dielectric constant strengthens the electrostatic interactions responsible for the salt effects, additionally decreasing the transition-state activity coefficient for both high- and low-charge density cations. However, in a sense, this is merely a restatement of the experimental observations.

On a molecular basis, the following tautomeric equilibria (based on an earlier proposal of Berger, *et al.*[11]) were put forward to account for the observed salt effects.[2] In this mechanism the presence of a high charge density cation would favor the formation of species D (by ion pairing with the negatively charged carbonyl oxygen), thereby increasing the total concentration of species B and D, both of which are unable to participate in the acid-catalyzed exchange process. On the other hand, it was proposed that cations of low charge density could form ion–dipole complexes with the partially negatively charged carbonyl oxygen of species C, resulting in complexes of type E and thus stabilizing the transition-state form (species C and E) which is catalytically active in the acid-catalyzed exchange process. We would expect that a decrease in solvent dielectric constant would stabilize such electrostatic interactions. Thus the addition of dioxane should increase the relative concentration of the catalytically inactive species D for high charge density cations, and thus reduce k/k_0 further (relative to the purely aqueous system). This is indeed observed. However, the effect of adding dioxane is also to decrease the observed k/k_0 values for the lower charge density cations. In terms of the above model this suggests that in the lower dielectric environment these cations also can form catalytically inactive ion-pair complexes of type D, partially offsetting the rate-enhancing effect of the formation of species E (*i.e.*, the stabilization of catalytically active species C).



As observed for acid-catalyzed exchange in the pure aqueous system,[2] the type of monovalent anion bound near the amide nitrogen appears to have very little effect on the exchange rate. This is in keeping with the model suggested here, since the detailed nature of the binding of the anion at the positively charged end of the amide dipole would have little effect on tautomeric equilibria between the species which are, and those which are not, catalytically active in the exchange process.

Base Catalysis Mechanisms. In contrast to the acid-catalyzed exchange process, base-catalyzed exchange proceeds by an altogether different pathway and would

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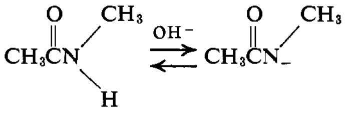
[11] A. Berger, A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, **81**, 62 (1959).

be expected[2] to show quite different patterns of neutral salt effects. The base-catalyzed exchange of the amide proton of NMA doubtless proceeds according to the following scheme.



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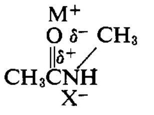
In this system ion–dipole interactions with species A would result in the following type of complexes with added electrolytes.



On this basis we would expect high charge density (tightly binding) cations to increase the partial negative charge on the carbonyl oxygen, increasing the basicity of the nitrogen and facilitating base-catalyzed proton exchange. This pattern is observed for Li^+, and would be expected to a lesser degree for the less highly charged cations. Anion binding at the partially positively charged nitrogen would be expected to augment this increase in basicity further. But in addition, and perhaps more importantly, the anion bound to the nitrogen might also be expected to repel the negatively charged hydroxide ion and thus *inhibit* the formation of the catalytically active species F. In other words, by this mechanism, the presence of the anion would be expected to *decrease* the effective basicity of the amide nitrogen, *i.e.*, to increase the pK for the proton dissociation reaction. And the more tightly bound the anion, the more effective it should be. It has been shown by a number of workers (see ref 1 for detailed citations) that the larger and more polarizable anions bind much more tightly than the smaller ones to amide dipoles. Thus, if the pK shifting effect described above plays the dominant role in the effect of anions on the base-catalyzed NMA exchange rate, we would expect (for a common cation) that k/k_0 would decrease as we increase the size and polarizability of the added anion. And, based on the above mechanism, we would expect that the cation-facilitated increase in exchange rate would increase (for a common anion) as we decreased the size of the cation. Table III shows that these expectations are indeed borne out: with the cation-induced increase in k/k_0 dominating the observed rates for the Li^+ salts (though progressively less so in the order LiCl > LiBr > LiI), the anion effects become progressively more dominant for the larger cations.

It should be pointed out specifically here that observed rate constants (Figure 3) for the base-catalyzed exchange process (k_{OH^-}) are still about three orders of magnitude less than those for a diffusion-controlled reaction, and thus we would expect effects on pK to swamp out any effects on the rates due to differences in the viscosities of the solutions. This is borne out by the fact that the measured relative viscosities of the ionic solutions used in this study (see Table I, ref 2) do not show any correlation with the rate effects summarized in Table III. This is also in keeping with the general conclusion of Noyes[12] that reaction rates are independent of solvent structure and viscosity effects for chemical reactions with second-order rate constants which are appreciably less than 10^7 M^{-1} sec^{-1}.

In conclusion, the data reported here seem to provide additional support for our previously proposed[2] model of salt effects on the acid-catalyzed rates of amide proton exchange. The effects on the base-catalyzed exchange process are quite different and seem compatible with a straightforward ion–dipole binding mechanism. Thus, in acid-catalyzed hydrogen exchange of proteins one would expect to see salt effects which depend primarily on the nature of the added cation, while for base-catalyzed protein hydrogen exchange in the presence of denaturing salts, as well as for nucleic acid hydrogen exchange,[13] one should expect salt effects which depend on both the nature of the cation and of the anion, as outlined in Table III. In addition to the practical consequences of these studies for macromolecular hydrogen exchange, these results also add molecular detail to our understanding of the nature of the amide–ion interactions which underlie the effects of neutral salts on the stabilities of macromolecular conformations.

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(12) R. M. Noyes, *Progr. React. Kinet.*, **1**, 129 (1961).

(13) See B. McConnell and P. H. von Hippel (*J. Mol. Biol.*, **50**, 317 (1970)) for a recent hydrogen exchange study of DNA conformation in the presence of high concentrations of destabilizing salts.