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Effect of Water Activity on the Rate of Proton Dissociation

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Abstract: The rate of proton transfer from electronically excited aromatic alcohols to the solvent was measured by both steady-state and time-resolved picosecond fluorimetry. In concentrated solutions (1-6 M) of strong electrolytes (LiBr, $LiClO_4$, NaCl, $NaClO_4$, KCl, $MgCl_2$, $MgClO_4$) at pH $\gg$ pK^*, the rates measured by the two methods were identical. The rate of dissociation decreases upon increasing the concentration of the salt. Results obtained with different salts fit a single straight line when the log of the rate constant is drawn vs. the log of water activity. Such measurements are suitable for accurate determination of $a(H_2O)$ of concentrated solutions of strong electrolytes. It is proposed that the rate of proton dissociation is related to the free energy of proton hydrate formation.

Introduction

In their first electronic excited singlet state, aromatic alcohols, or amines, are stronger acids than in their ground state (p$K^* \ll$ pK^0).[1,2] The fluorescence spectra of such compounds consists of two emission bands. The band with the shorter wavelength is the emission of the neutral form (ROH*) while the other one, at the longer wavelength, is that of the excited anion (RO^{-*}). The energy difference between the two bands is proportional to the pK shift, as described by the Forster cycle.[3]

Time-resolved fluorescence, carried out on picosecond time scales,[4] revealed the correlation between the thermodynamic property of the excited compounds (pK^*) and the rate of proton dissociation. Strong acids, like excited 2-naphthol-3,6-disulfonate (pK^* = 0.5), ejects its proton to the water at a rate of 1.4×10^{10} s^{-1} while a weaker acid like 2-naphthol (pK^* = 2.5) dissociates at a rate of 3×10^8 s^{-1}.

In contrast to the fast dissociation in water, in other solvents (methanol, ethanol, propanol, glycerol, formamide) no dissociation takes place, even if the dielectric constant of the solvent is higher than that of water (formamide, $\epsilon = 109.5$). in water-alcohol mixture the rate of dissociation decreases with the mole fraction of the water. This correlation was explained[5,6] in terms of the effect of alcohol on the organization of the water molecules and their ability to stabilize the ejected protons, as described below.

Proton dissociation can be regarded as a two-step reaction. The first step is dissociation from the parent molecule to form a thermodynamically stable hydronium ion, followed by the diffusion of the proton out of the Coulomb cage, where recombination is extremely fast.[7] Any event which will either destabilize the hydronium ion with respect to the parent molecule or retard it in the Coulomb cage will slow the overall rate. The presence of alcohols in the solution interferes with both processes by affecting the tendency of water molecules to form tetrahedrally oriented hydrogen-bridge regions.[8,9] As is known from gas-phase studies of water clustering around protons[10,11] and from quantum-mechanical calculations.[12,13] the stability of the proton hydrate complex increases with the number of water molecules in the cluster. Thus, by affecting the size of the hydration complex, alcohols will lower the free energy change of the proton transfer to the solvent and destabilize the dissociated state with respect to the parent molecule.

The proton escape out of the Coulomb cage might also be hindered by the alcohols. The rapid motion of the proton between the water molecules of the hydronium complex[14] is an effective escape route out of the Coulomb cage.[6] Once the size of the hydration complex is shrunk (by the alcohol), the probability of rapid escape will be affected.[5,6]

In our previous publications[5,6] we demonstrated a free energy relationship for the kinetics of proton transfer from various donors to the same acceptor, H_2O. In the present study we expanded these free energy relationship studies by varying the chemical potential of the acceptor—the water.

The water-alcohol mixtures described above[5,6] are not convenient for measuring the role of water in the proton dissociation reaction. The large Coulomb cage (28 Å for 8-hydroxypyrene-1,3,6-trisulfonate (HPTS))[7,15] and its expansion upon lowering of the dielectric constant introduce a nontrivial contribution of the ion-pair recombination to the observed reaction rate. Concentrated solutions of strong electrolytes are a much better system, as at concentrations above 1 M the electrostatic screening shrinks the Coulomb cage to about the molecular diameter of HPTS. This effective electrostatic screening practically eliminates the role of the Coulomb cage in the recombination. The dissociative step itself will already place the proton out of the range of the electrostatic attraction. Under such conditions we can study the role of water activity in proton dissociation.

Experimental Section

The proton emitter compounds used in this study, 2-naphthol, 2-naphthol-6-sulfonate, 2-naphthol-3,6-disulfonate, 2-naphthol-6,8-disulfonate, 8-hdyroxy-1,3,6-pyrenetrisulfonate, and the pH indicator bromocresol green were reagent grade and used without further purification. The inorganic electrolytes NaCl, $MgCl_2$, LiBr, $LiClO_4$, $NaClO_4$, and $Mg(ClO_4)_2$, reagent grade, were dissolved in triple-distilled water.

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(15) Abbreviations: HPTS, 8-hydroxypyrene-1,3,6-trisulfonate; BN6S, β-naphthol-6-sulfonate. k_{off}, k_{on}: the first-order rate constant of proton dissociation and the second-order rate constant of proton recombination of the excited proton emitter. ϕ, ϕ_0: measured and maximal fluorescence quantum yield of ROH. ϕ', ϕ_0': measured and maximal fluorescence quantum yield of RO^-.

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Table I. Proton Transfer Rates (k_{off}) from Excited HPTS in Various Concentrations of Aqueous Salt Solutions[a]

$LiClO_4$		$MgCl_2$	
salt concn, mol/L	$10^{-9}k_{\text{off}}$, s^{-1}	salt concn, mol/L	$10^{-9}k_{\text{off}}$, s^{-1}
0	10	0	10
0.95	5	0.1	8.4
1.9	2.5	0.2	5.7
2.7	1.3	0.57	5
3.1	1.05	1.7	1.8
4.1	0.55	2.29	0.95
5.9	0.19	2.86	0.54
		3.43	0.24
		3.89	0.16

[a] The error limits are ±15%.

The pH was lowered by 10^{-5} M (final concentration) of perchloric or hydrochloric acid. Steady-state fluorescence was measured by using a Perkin-Elmer Hitachi MPF-4 spectrofluorometer. For kinetic studies the sample, held in a 1-mm optical path quartz cuvette, were excited by a 6-ps pulse of the third harmonic frequency of a Nd^{3+}/glass laser (352 nm). The decay time of the fluorescence was measured by a Hammamatsu C939 streak camera combined with an optical multichannel analyzer (PAR 1205D). The fluorescence decay times and rise times are accurate to ±15% provided first-order decay is obeyed. The wavelength of the fluorescence was selected by cutoff filters and narrow-band interference filters. For further details see ref 5 and 6.

Results

Effect of Concentrated Salt Solutions on the Rate of Proton Dissociation. In concentrated solutions of strong electrolytes ($MgCl_2$, $LiClO_4$), the decay time of the fluorescence of the undissociated HPTS[15] (ROH*) is much longer than the decay time measured in water (Figure 1). Under conditions where recombination of the proton with the excited emitter can be neglected (pH $\gg$ pK^*), the rate of proton dissociation is calculated from the decay time by using expression 1[4-6] or from steady-state

$$k_{\text{off}} = \frac{\tau_0 - \tau_{\text{obsd}}}{\tau_0 \tau_{\text{obsd}}} \qquad (1)$$

fluorescence measurements by using the equation given by Weller[16]

$$\frac{\phi/\phi_0}{\phi'/\phi_0'} = \frac{1}{k_{\text{off}}\tau_0} + \frac{k_{\text{on}}\tau_0'}{k_{\text{off}}\tau_0}[\text{H}^+] \qquad (2)$$

where k_{off} is the proton transfer rate, k_{on} is the recombination rate, τ_0 is the lifetime and ϕ_0 is the quantum yield of the neutral species in the absence of a proton transfer process, and ϕ_0' is the quantum yield and τ_0' is the lifetime of the anionic species being excited from its ground state RO^-. ϕ' is the quantum yield of RO^{-*} prepared from ROH via the transfer of a proton from ROH*. ϕ is the quantum yield of ROH* when proton transfer occurs.

Typical fluorescence decay curves of the neutral form of HPTS are shown in Figure 1; the proton transfer rates obtained for various concentrations of $MgCl_2$ and $LiClO_4$ in aqueous solution are listed in Table I. Comparisons between rates of proton dissociation calculated from either kinetic or steady-state fluorescence measurements are given in Figure 2. The agreement between the two methods is good only if the proton does not recombine with the excited anion before it decays to the ground state. In most cases, where pH $\gg$ pK^*, no such recombination occurs and steady-state fluorimetry can be used for measurements of k_{off}. Still, this assumption must be experimentally verified. Under certain conditions, such as reaction in a microcavity in a protein, a rapid proton recombination was observed.[17]

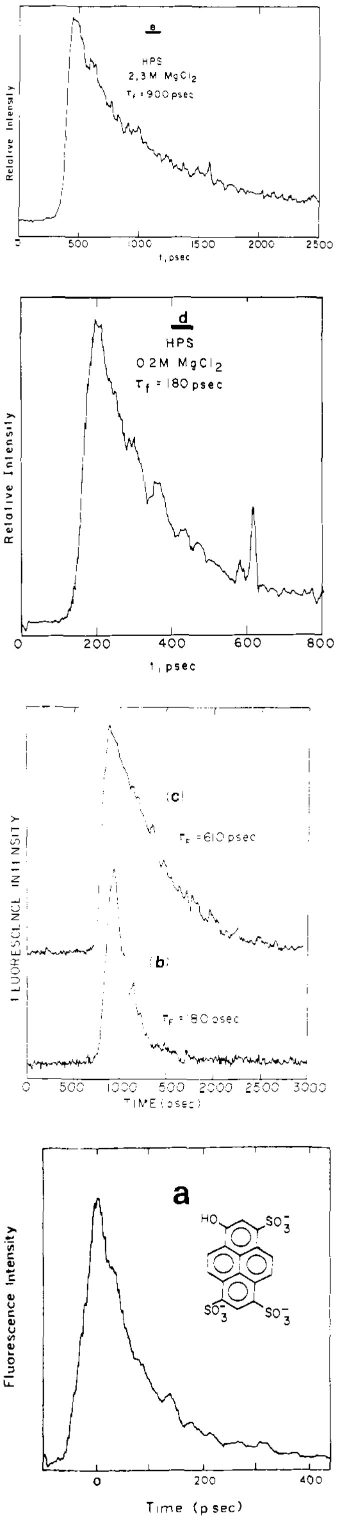

Figure 1. Fluorescence decay curves of the neutral form of 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) measured at the spectral range 400–470 nm in water, water–$LiClO_4$, and water–$MgCl_2$ solutions; (a) pure water; (b) solution containing 1 M of $LiClO_4$; (c) solution containing 2.5 M of $LiClO_4$; (d) solution of 0.2 M $MgCl_2$; (e) solution containing 2.29 M $MgCl_2$.

Figure 3a relates the measured rate of proton dissociation with the molar concentration of the salts used in the experiment, NaCl, LiBr, and $MgCl_2$. Each salt is characterized by a different curve, but, if the rates are drawn vs. the water activity in these solutions (calculated from the water vapor pressure[19]), a straight relationship is obtained (Figure 3b).

A linear correlation between the measured rate and $a(H_2O)$ (on a log–log scale) has been measured for five more compounds

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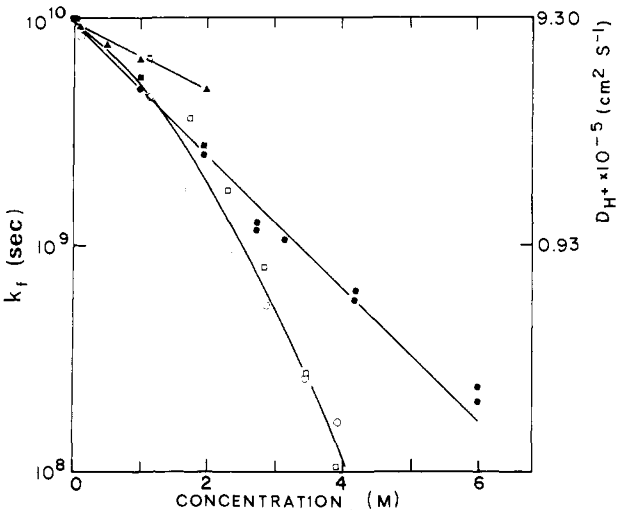

Figure 2. Semilog plot of the dependence of the proton transfer rate on the electrolyte concentrations (in molar units): (circles) time-resolved fluorescence measurements; (squares) proton transfer rates determined from steady-state fluorescence measurements; (triangles) the proton diffusion constant measured by electrochemical techniques taken from ref 18. Solid symbols: water–$LiClO_4$ solutions. Open symbols: water–$MgCl_2$ solution.

whose k_{off} values (measured in pure water) differ by 5 orders of magnitude (Figure 4). The slopes of the lines vary with the structure of the compound. Those, which do not have a hydrophylic moiety in proximity to the dissociating proton tend to have steeper slopes. In addition to the study of proton transfer in the excited state, we have extended the measurements to the ground state. Utilizing the pH jump method,[20] we have determined the proton transfer rate of ground-state bromocresol green. As seen in Figure 4, the dependence of the rate on the water activity is similar to that of excited HPTS. Note that the transfer rates of bromocresol green are about 5 orders of magnitude smaller than those for excited HPTS.

Determination of $a(H_2O)$ of Concentrated Solutions by K_{off} Measurements. The results presented in Figure 4 are a set of calibration lines measured with six different compounds. Each line relates the rate of proton dissociation with respect to water activity. In order to check whether we can use this technique for estimating $a(H_2O)$ of electrolyte solution, we measured the rate of proton dissociation from two compounds (HPTS and BN6S) dissolved in NaCl solution (0–4.5 M). As demonstrated in Figure 5, $a(H_2O)$ of those solutions, calculated from vapor pressure data,[19] does not differ (within experimental accuracy) from the values obtained by the fluorometric technique. The reliability of these measurements is further emphasized in Figure 6. In this figure $a(H_2O)$ of various solutions, $Mg(ClO_4)_2$ (0–3 M) and $NaClO_4$ (0–4 M), as measured by one compound (HPTS) is drawn vs. the values measured by using the other one (BN6S). It is evident that, within our experimental accuracy, both compounds yield identical results.

Discussion

In the present study we describe the effect of strong electrolytes on the rate of proton dissociation in aqueous solutions. This overall reaction consists of three partial reactions all taking place in the Coulomb cage: ion-pair formation, ion-pair recombination, and ion-pair separation (escape of the proton our of the Coulomb cage). The rate constants of these three events contribute to the measured rate constant. As all these steps are associated with charge separation (or recombination), they are strongly affected by electrostatic forces. Thus, in the first section of the discussion we shall examine whether our observation can be explained in terms of electrostatic effects.

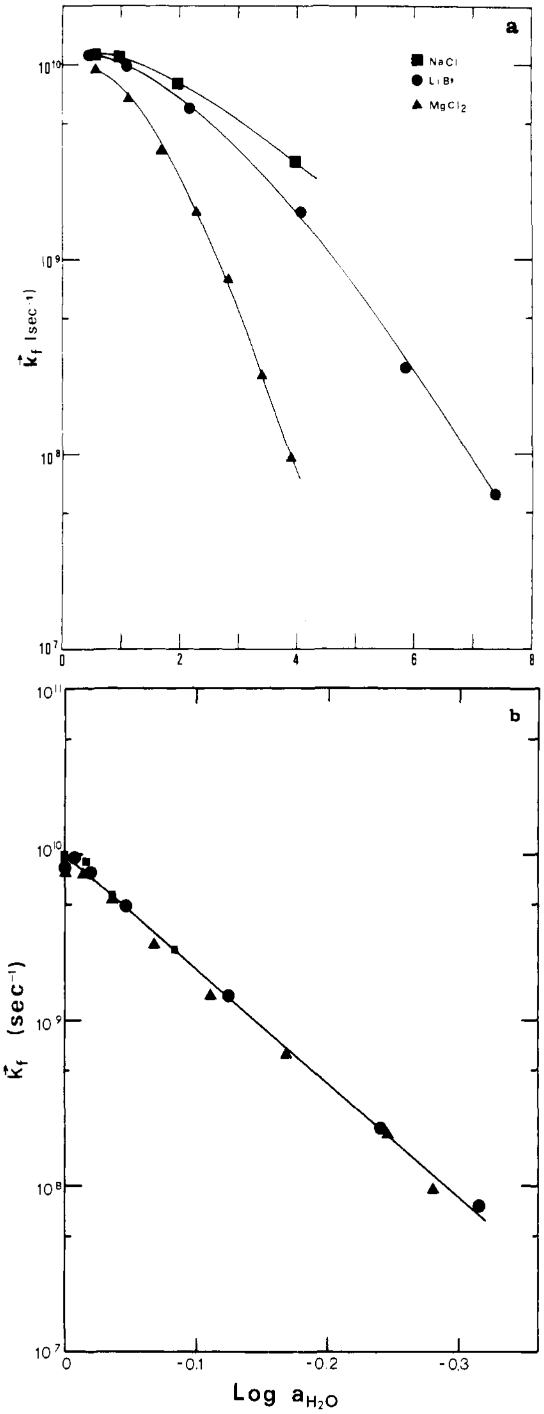

Figure 3. (a) Proton transfer rate from excited HPTS in aqueous solution containing electrolytes as a function of the electrolyte molar concentration: (●) NaCl; (▲) LiBr; (■) $MgCl_2$. (b) log–log plot of the dependence of proton transfer on the activity of water. The water activity is decreasing when electrolyte concentration is increased. Note that the data points are taken from Figure 3a.

The first event, ion-pair formation is a short–range reaction which is not subjected to ionic screening. The effect of electrostatic interactions on the two other reactions, ion-pair recombination and ion-pair separation, were calculated by Eigen et al.[21]

In concentrated salt solutions, a considerable fraction of the water molecules are oriented in a hydration shell around the ions; thus, their dielectric constant is smaller than in pure water.[22] A decreased dielectric constant will accelerate ion-pair recombination

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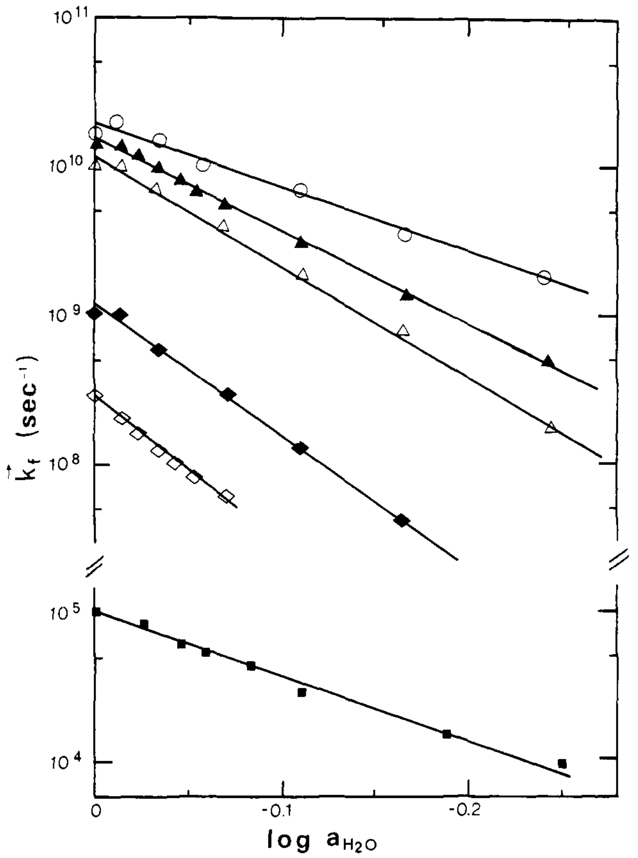

Figure 4. Dependence of the proton transfer rate on water activity for various excited hydroxy aromatic compounds: (○) 2-naphthol-3,6-disulfonate; (▲) 2-naphthol-6,8-disulfonate; (△) 8-hydroxy-1,3,6-pyrenetrisulfonate; (◆) 2-naphthol-6-sulfonate; (◇) 2-naphthol; (■) bromocresol green in the ground state. Note the discontinuity of the ordinate.

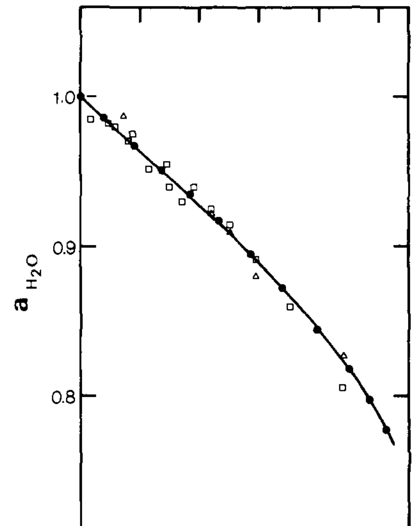

Figure 5. Effect of NaCl concentration on the activity of water in the solution. $a(H_2O)$ was calculated from vapor pressure data (ref 19) (●) or as estimated by the rate of proton dissociation from HPTS (△) or BN6S (□).

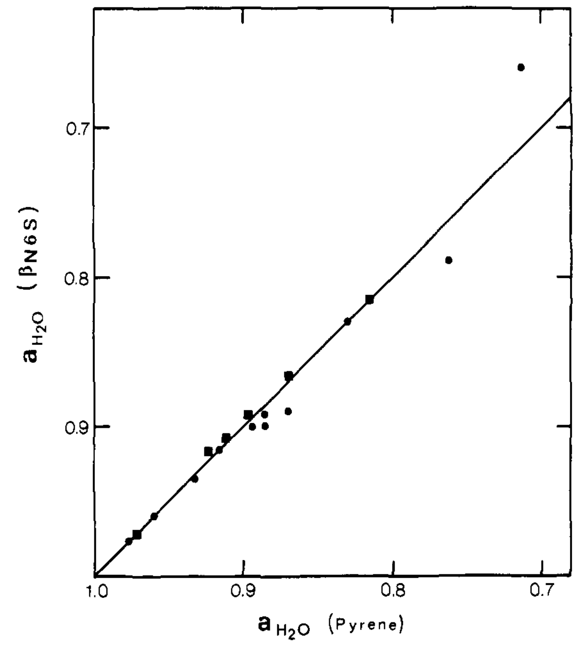

Figure 6. Correlation between $a(H_2O)$ of concentrated solutions of $Mg(ClO_4)_2$ (●) and $Na(ClO_4)$ (■) calculated from the rate of proton dissociation from HPTS (abscissa) and BN6S (ordinate).

and slow down ion-pair separation. The combination of these two effects will lower the probability of proton dissociation in accord with our observation. Yet, this explanation is not applicable for our case. An appreciable decrease of the solution's dielectric constant occurs at very high concentrations (above 1 M of electrolyte). At such high concentrations the ionic atmosphere will effectively screen the proton from the electric charge of the parent molecule.[23] In such concentrated solutions where the radius of the ionic atmosphere is shorter than that of the Coulomb cage, the rigorous analysis of Eigen is not applicable anymore.[21] Even if precise calculations are not available, it can be argued that electrostatic screening cannot explain our observations: the ionic screening (primary salt effect) will slow the ion-pair recombination with no effect on ion-pair separation. The combination of these two effects will acclerate the overall rate of proton dissociation, which is in contrast with our observations.

As was demonstrated before,[5,6] the decrease in the diffusion coefficient of protons in ethanol-water mixture is too small to account for the observed decrease in the rate of dissociation. Thus, the escape of the proton out of the solvent cage is not the rate-limiting step in the dissociation. The same conclusion is also applicable for the present case. As depicted in Figure 2, the decrease of the diffusion coefficient of H^+ in concentrated salt solution is insufficient to account for the slowed-down dissociation. Thus, the rate-limiting step in our observation is not the diffusion of the proton.

Apparently neither electrostatic interactions nor reduced diffusibility of protons is the major cause for the decrease in the proton transfer rate. As these effects are dominating in ion-pair recombination and ion-pair separation, we have to focus our attention to the primary event in proton dissociation, ion-pair formation. In this reaction the hydrogen of the O–H bond of the excited parent molecule forms a hydrogen bond with the nearest H_2O molecule which itself is hydrogen bonded to nearby water molecules. If the proton moves by ~0.5 Å along the line connecting it to the nearest H_2O, the O–H bond breaks, and H_3O^+ is formed. The enthalpy of proton hydration is 270 kcal/mol, while the enthalpy of formation of H_3O^+ is estimated to be 170 kcal/mol.[24] The energy difference of ~100 kcal is attributed to further solvation of H_3O^+ by additional water molecules. Within the time frame of proton dissociation (probably comparable to OH vibration or water libration time), a stable hydronium must be formed, otherwise, the proton will revert to the parent molecule. Molecular dynamic simulations indicate that the formation of

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(23) A 1 M solution of univalent electrolyte like NaCl will lower the dielectric constant by 10%. At this concentration the radius of the ionic atmosphere will be 3.1 Å.

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hydration shell might be a subpicosecond event.[25] Just because the stabilization of the H_3O^+ in the hydration complex is of paramount importance in the dissociation event, any perturbation at this step might be crucial for the rest of the reaction to occur.

Figure 3a demonstrates that the effect of salts on the rate of proton dissociation is not a simple function of the salt concentration. The rate measured in equimolar concentrations of NaCl, LiBr, or $MgCl_2$ varied markedly. Other concentration parameters of the solution such as molality or mole fraction were of no further advantage. On the other hand, the function which reflects the properties of the solvent in the solution, the activity of H_2O, was found suitable: Experimental results obtained with the three electrolytes fit a single linear function

$$\log k' = \log k_0 - n \log a(H_2O) \qquad (3)$$

where k' and k_0 are the proton transfer rates in salt solutions and in pure water, respectively.

Equation 3 is compatible with a reaction mechanism where the excited molecule transfers a proton to a hydration complex of n water molecules. Such a presentation is a gross oversimplification as n becomes a stoichiometic factor which states that no reaction will take place with the species $(H_2O)_{n-1}$ or $(H_2O)_{n+1}$. Thus, a less stringent explanation should be looked for.

Searcy and Fenn[10] and Kebarle[11] measured the clustering of water molecules around free protons in the gas phase. Clusters with varying size were observed and the respective enthalpy of formation was calculated. The difference in enthalpy of hydration of a proton vs. the cluster number n, designated as $-\Delta H°_{n,n+1}$, shows a remarkable decrease while increasing the cluster number n. The hydration enthalpy difference between a monomer H_3O^+ and a dimer is $-\Delta H°_{1,2}$ = 32 kcal/mol, while $\Delta H°_{2,3}$ = 22 kcal/mol. These values are comparable with the results obtained by quantum-mechanical calculations.[12,13] The hydration enthalpy $-\Delta H°_{n,n+1}$ is reaching a limiting value of about 10 kcal/mol when the cluster number is about 10. By analogy with these results, the enthalpy of proton hydration in solution will also increase with the size of the hydration complex. Yet, in liquid water one exception should be made: In order to increase the size of the complex by one water molecule, a water molecule must first be removed from the bulk, with energy investment of 10 kcal/mol (heat of evaporation of water). Therefore, the hydration complex of a proton will not exceed the state where the energy gain of further hydration will be comparable with the heat of evaporation. Using the results of Kebarle[11] and Searcy and Fenn,[10] we estimate that the hydrating complex in dilute electrolyte solution ($a(H_2O)$ = 1) will be of 10 water molecules, or less. The above conclusion bears directly on our observation.

In concentrated salt solutions, the vapor pressure is lower than that of pure water and hence it exhibits reduced water activity. This phenomenon is explained by the fact that a considerable fraction of the water molecules are associated with the hydration of the salt ions. The binding energy of these water molecules (which form the first and second hydration shells), to the center ion, is larger than 10 kcal/mol. Therefore, they are less likely than the free water molecules to participate in the process of the hydration of the initially formed H_3O^+. In order to obtain a proton hydrate greater than H_3O^+, the thermodynamically stable complex must be formed within the ion-pair lifetime. The depletion from the solution of water molecules available for this reaction will lower the probability of the successful dissociation. As demonstrated in Figure 5, indeed this function decreases with the activity of the water in the solution.

Finally, we wish to demonstrate the applicability of this technique for estimation of $a(H_2O)$, in contrast to the usual techniques based upon colligative properties or emf measurements of electrochemical cells. The values calculated from measurements using different proton emitters are practically identical (Figure 6) and are comparable with values calculated by vapor pressure studies (Figure 5). The applicability of this method for estimation of the equivalent water activity in the microspace of an active site of a protein has already been demonstrated.[17]

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Registry No. H_2O, 7732-18-5; 2-naphthol-6-sulfonic acid, 93-01-6; 2-naphthol-3,6-disulfonic acid, 148-75-4; 2-naphthol-6,8-disulfonic acid, 118-32-1; 8-hydroxyl-1,3,6-pyrenetrisulfonic acid, 27928-00-3; 2-naphthol, 135-19-3.

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Intramolecular Hydrogen Bonding in Gramicidin S. 2. Ornithine

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Contribution No. 6611 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125. *Received March 1, 1982*

Abstract: The conformation of the ornithine side chains in gramicidin S (GrS) in solution was investigated by ^1H and ^{15}N NMR spectroscopy at 11.7 T. Rotational averaging of the chemical shifts of the Orn $C^\delta H_2$ protons was incomplete, the degree to which the apparent motility of the side chain is limited varying inversely with the ability of the solvent to compete for hydrogen-bonding (H-bonding) donor or acceptor sites. Methylation of GrS to give [2,2′-N^δ-trimethylornithyl]GrS resulted in an upfield shift of 3.5 ppm in the ^{15}N resonance of Pro in MeOH and abolished the correlation of the Orn $C^\delta H_2$ splitting with solvent basicity. The data are consistent with the presence of intramolecular Orn $N^\delta H_3^+$--O=C D-Phe H bonds, each with formation constant ~1.1 in MeOH at 23 °C, and exerting a substantial charge relay effect on the Pro ^{15}N chemical shift. Thermodynamic analysis of the Orn $C^\delta H_2$ proton splitting yielded estimates of $-\Delta H° = 2.3 \pm 0.4$ kcal mol^{-1} and $-\Delta S° = 7.5 \pm 1.0$ cal deg^{-1} mol^{-1} for the transition of each residue from the inter- to the intramolecularly H-bonded configuration in MeOH and +10.1 ppm for the total charge relay shifted at Pro ^{15}N. Proton exchange kinetics and NOE measurements indicate that the H bonds are formed in the $i \rightarrow i + 2$ sense. Estimates for the Orn side chain torsional angles in the intramolecularly H-bonded configuration are given, and the possible origin of the Orn $C^\delta H_2$ chemical shift inequivalence is discussed. The possible functional role of the H bonds is considered.

The cyclic decapeptide antibiotic gramicidin S (GrS; *cyclo*-[Val^1-Orn^2-Leu^3-D-Phe^4-Pro^5)$_2$, Figure 1a) contains, as do a number of other biologically active peptides, cationic amino acid side chains which are essential for activity.[1] While considerable