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Studies in Solvation. 5. Activation Parameters for the Imidazole-Catalyzed Hydrolysis of *p*-Nitrotrifluoroacetanilide. Entropy Control in Catalysis by Water[1,2]

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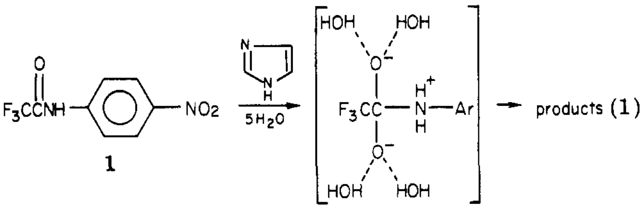
We previously reported that the rate of imidazole-catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide showed a high order in water in mixtures of water and acetonitrile.[2a] We now report that the activation parameters have been determined at four water concentrations and that the large rate increases from catalysis by water are due to changes in ΔS^*, not ΔH^*. These results are applied to understanding solvation of the transition state and are related to enzymic catalysis.

This study is part of our investigations of solvation by water along reaction pathways,[2] a problem that not only has intrinsic importance but also has application to understanding the catalytic roles of functional groups at the active sites of enzymes because the close fit between enzyme and substrate probably demands that solvent is excluded from the active site when substrate is bound. Direct support for this view has been found recently in crystallographic studies of kinases;[3] these enzymes undergo a large conformational change on substrate binding. It appears that this produces a very close fit between enzyme and substrate which leaves no room for water molecules.[4] This appears to have the advantage of preventing the inefficiency of a side reaction: phosphorylation of water.[5] Therefore, in order to understand fully the fundamental chemistry of both enzymic reactions and reactions in aqueous solutions, one needs to know the role of water in the reaction.

The imidazole-catalysed hydrolyses of *p*-nitrotrifluoroacetanilide (pNTA, 1) and similar amides have been studied as analogues to the hydrolyses of peptide bonds.[2,6-9] We studied the rate of this reaction in acetonitrile as a function of water concentration; we found that the rate decreased rapidly as [H_2O] decreased. The dependence of the observed rate constants on [H_2O] indicated a high order in water that we attributed to solvation of a highly polar tetrahedral intermediate formed by proton transfer from the initially formed tetrahedral intermediate (eq 1).[2a] In order to understand this thermodynamically, we have determined the activation parameters as a function of water concentration.



Experimental Section

p-Nitrotrifluoroacetanilide (1) was prepared as described in our earlier paper.[2] Imidazole was recrystallized twice from benzene and once from benzene–heptane, mp 89–90.5 °C (lit. mp 88–90 °C). Perchloric acid was determined to be 69.9% (w/w) by titration. Acetonitrile was Mallinckrodt spectrophotometric grade stored over molecular sieves. Water was distilled, boiled for 15 min to remove CO_2, and stored under Ascarite.

Solutions, which consisted of 1.0 M imidazole, 0.1 M imidazolium ion, 1×10^{-4} M pNTA, water, and acetonitrile, were prepared with all components except pNTA, and were equilibrated in a constant-temperature bath for at least 15 min. pNTA was added in two different ways: for most of the project 0.05 mL of a 2.0×10^{-2} M solution was added to the 10-mL volumetric flask with a syringe and the solution was mixed before being placed in a cuvette; in later reactions 0.015 mL was added, by syringe, to 3.0 mL of reaction solution in the cuvette.

Rates of reaction were followed spectrophotometrically at 380 nm and the data analyzed to obtain pseudo-first-order rate constants.[10] Temperatures in the Cary 16's sample compartment were measured with a calibrated thermometer with graduations at each 0.1 °C. Reactions were followed for 10–12 h, unless the infinity point, taken to be the absorbance at 10 half-lives, occurred sooner.

At 68.8 °C, we were concerned about the volatility of acetonitrile (bp 81 °C); therefore, the hydrolysis was run in ampules. Three-plus milliliters were sealed in each of 12 ampules that were heated in a thermostated oil bath. In slow reactions, we noted that there was not a sufficiently stable infinity point, probably because of oxidation of *p*-nitroaniline. Therefore, we used the following methods: for reactions that were complete in 10–12 h, the rate constant was calculated, with use of the experimental infinity point, by the least-squares method.[10,11] However, when this was not possible, a nonlinear estimation program that calculates theoretical end points was used. The accuracy of this program was checked by taking data from a run with a known endpoint and calculating the rate constant by both methods; the rate constants differed by less than 2% and the endpoints by less than 1%. The nonlinear estimation method utilizes an iterative fit to the data by using Taylor's expansion through the first derivatives. Least-squares determination yields differences between estimated and best-fit values. These differences are added to the estimated values in each iterative cycle. From large numbers of applications of this method, it appears to be more

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[2] Previous publications: (a) J. W. Henderson and P. Haake, *J. Org. Chem.*, **42**, 3989 (1977); (b) G. Wallerberg and P. Haake, *ibid.*, **46**, 43 (1981); (c) G. W. Allen and P. Haake, *Bioorganic Chem.*, **9**, 325 (1980); (d) J. Bonicamp and P. Haake, *Tetrahedron Lett.*, in press.

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Table I. Rate Constants for Reaction of pNTA (1)[a]

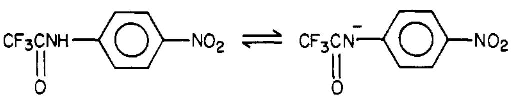
$[H_2O]$, M	T, °C	k_{obsd}, min^{-1}	k_{obsd}^{corr}, min^{-1}
5.3	43.9	2.16×10^{-3}	2.21×10^{-3}
	48.8	2.89×10^{-3}	2.95×10^{-3}
	48.8	2.71×10^{-3}	2.76×10^{-3}
	50.6	3.46×10^{-3}	3.52×10^{-3}
	54.5	4.30×10^{-3}	4.39×10^{-3}
	58.6	6.12×10^{-3}	6.24×10^{-3}
	64.9	7.04×10^{-3}	7.18×10^{-3}
	68.8	8.96×10^{-3}	9.14×10^{-3}
15	29.1	1.90×10^{-3}	2.00×10^{-3}
	38.2	3.67×10^{-3}	3.85×10^{-3}
	43.9	5.31×10^{-3}	5.58×10^{-3}
	48.8	6.93×10^{-3}	7.27×10^{-3}
	50.7	8.12×10^{-3}	8.53×10^{-3}
	54.5	1.12×10^{-2}	1.17×10^{-2}
	58.6	1.41×10^{-2}	1.48×10^{-2}
30	29.9	6.06×10^{-3}	6.45×10^{-3}
	37.7	1.28×10^{-2}	1.39×10^{-2}
	43.9	1.63×10^{-2}	1.76×10^{-2}
	48.8	2.27×10^{-2}	2.46×10^{-2}
	51.4	2.86×10^{-2}	3.09×10^{-2}
	54.5	3.29×10^{-2}	3.55×10^{-2}
	58.6	4.39×10^{-2}	4.74×10^{-2}
52	19.5	1.94×10^{-2}	4.04×10^{-2}
	29.1	4.16×10^{-2}	8.69×10^{-2}
	38.2	7.70×10^{-2}	1.61×10^{-1}
	38.2	7.29×10^{-2}	1.52×10^{-1}
	43.9	1.16×10^{-1}	2.43×10^{-1}
	48.8	1.58×10^{-1}	3.30×10^{-1}

[a] 1.0 M imidazole, 0.1 M imidazolium ion.

reliable than other methods of determining the infinity point.

Results

The reactions were carried out in the presence of a buffer, 1.0 M imidazole, and 0.1 M imidazolium ion, in order to avoid a HO^- component in the rate.[2] All reactions followed pseudo-first-order kinetics. The rate constants were corrected for dissociation of pNTA to its unreactive anion (eq 2),[2] giving corrected rate constants (Table I), k_{corr} = k_{obsd} (neutral pNTA/total pNTA). In order to determine correction factors, UV–vis spectra of reaction solutions were taken corresponding to solutions of the product, 100% anion (in 0.1 M NaOH), and 100% neutral (in 0.1 M $HClO_4$) solutions. The λ_{max} of the 100% anion solution was used as a common wavelength for all calculations, with absorbances extrapolated to t_0 in media where reaction was so fast that this correction was required. Beer's Law was then solved for the concentration of neutral pNTA. Correction factors were found to be 2.09 for 52 M water, 1.08 for 30 M water, 1.05 for 15 M water, and 1.02 for 5 M water.



(2)

neutral pNTA
1

anionic pNTA

Activation parameters have been calculated at each of the water concentrations mentioned from plots of ln (k_{corr}) vs. $1/T$ (°K) and from a least-squares program.[10] The final values of ΔG^*, ΔH^*, and ΔS^* were calculated for 43.9 °C. Together with standard deviations they are shown in Table II.

It was found that the free energy of activation decreases as the water concentration increases. The enthalpy remains remarkably constant. The change in ΔG^*, and hence in the rate of reaction, seems entirely due to the change in the entropy of activation. The changes in ΔH^* may be within experimental error; the changes in ΔS^*, and especially the trend, appear to be significant,[11] especially because of the large range and number of temperatures used for each water concentration.

Table II. Activation Parameters for 43.9 °C[a]

$[H_2O]$, M	ΔG^*, kcal/mol	ΔH^*, kcal/mol	ΔS^*, cal (mol·deg)
5	25.0	11.9 (1.4)	−41.4 (4.4)
15	24.4	12.8 (1.0)	−36.7 (3.0)
30	23.7	12.6 (1.2)	−35.0 (3.9)
52	22.1	12.7 (0.9)	−29.6 (2.8)

[a] Standard deviations are in parentheses.

Discussion

On the basis of previous research,[6–9] the mechanism of the reaction (eq 1) involves general-base catalysis by imidazole, which potentiates the nucleophilicity of a water molecule. Therefore, one water molecule is required as a nucleophile in the reaction. In our previous paper, we found evidence for additional solvation of transition state over ground state by four water molecules (eq 1).[2a] We attributed this to the need for protic solvation of the highly polar transition state which is required because of the difficulty in breaking the C–N bond.

We have only studied activation parameters at water concentrations of 5 M and greater because we previously found a sharp decrease in rate at water concentrations below 1 M, probably because most of the H_2O is complexed to imidazole and unavailable for solvation. Our previous paper[2a] details the reasons that the same mechanism of reaction is expected throughout the range of water concentrations that we have used in this study of activation parameters.

Acetonitrile is a particularly useful solvent for these studies because it is completely miscible with water, it has a high dielectric constant, it has a very high dipole moment (approximately 4 D), and it is a very weak acid and base: the strong dipole and high dielectric indicate that change of solvent composition will not greatly change the long-range solvent effects on solutes. The poor acid and base qualities mean that hydrogen bonding will be weak so essentially all hydrogen bonding with the substrate and transition state will be due to the water in the medium.

The final results in Table II provide quite strong evidence that there is no change in the energy of activation (and therefore in ΔH^*). Rather, there is an entropy barrier as $[H_2O]$ decreases in the medium. Interpretation of the results can be based on two hypotheses: First, as water increases in the medium, the transition state could become more highly solvated. Second, the transition state could have the same degree of solvation throughout the range of water compositions and the rate effects could be due to the smaller number of ground states with adequate solvation to form the transition state. We suggest that the latter hypothesis is supported by the data in this and the previous[2] papers for the following reasons: We found a reasonably constant dependence of log k (observed) on log $[H_2O]$ over a wide range of water concentrations; this suggests that the order in water remains constant and that the solvation difference between ground state and transition state is constant. If the solvation of the transition state were changing, one would expect the energy of the transition state to change, and this should result in a change in ΔH^*. But Table II demonstrates that ΔH^* is invariant with water concentration. This result is consistent with a transition state of reasonably constant structure and solvation. That is, only the intermediate shown in eq 1 leads to products; however, as the amount of water decreases in the medium, the concentration of that intermediate decreases; other intermediates need to be transformed into that intermediate before product can be formed. So this phenomenon becomes essentially a probability effect and it shows up in the entropy part of the activation parameters. Part of the problem may be reversion of intermediates to starting materials in competition with intermediates forming product. Higher solvation is required to form products as discussed previously.[2a] One can also describe the entropy barrier as a decreased probability of adequate numbers of water molecules in the solvation shell of the intermediate that forms the product transition state.

In contrast to eq 1, Bender and Komiyama have concluded from a proton-inventory study that solvation of the pNTA reaction requires only two water molecules.[6] Because of the assumptions inherent in the equation which is the basis of the interpretation of isotope effects,[12] on which the proton-inventory method is based, we tend toward the view that our simple method based on rate effects in acetonitrile-water may have greater reliability in assessing solvation. However, the principle finding in this paper, *entropy control*, is independent of the exact number of waters involved in solvation of the transition state.

A major part of enzymic catalysis appears to be due to the lack of an entropy barrier: the "Circe effect".[4] Our observation in this paper of the entropy barrier to solvation of transition states indicates that functional groups on enzymes will not only play critical roles such as that of nucleophile but they will also have to solvate the intermediates and transition states along the reaction pathway. In the function of enzymes, a knowledge of the way the amino acid residues are involved in solvating the substrate must be a major part of the total understanding of enzymic binding and catalysis.

These results also demonstrate that water as a solvent provides a minimum energy pathway to products in many reactions in a way that is somewhat similar to the mechanism of action of enzymes. That is, the acidic and basic properties of water provide catalysis and solvation with little entropy barrier when a reaction is carried out in pure water.

Registry No. 1, 404-27-3; imidazole, 288-32-4.

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Influence of Water Concentration in Acetonitrile upon the Transition-State Structure for 1-Acetyl-3-methylimidazolium Ion Hydrolysis[1]

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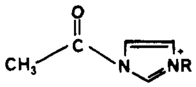
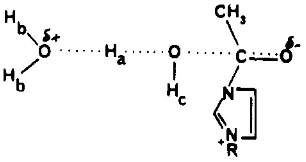
The hydrolysis of 1-acetyl-3-methylimidazolium ion has been investigated in water and water–acetonitrile mixtures with the proton-inventory technique. The influence of the solvent composition upon the β-deuterium secondary isotope effect and upon the thermodynamic activation parameters has been investigated as well. The relatively large solvent deuterium isotope effects (k_{H_2O}/k_{D_2O}) of about 2.5–2.8 at water concentrations greater than 1 M in acetonitrile were shown to be consistent with a transition-state structure in which one water molecule acts as a general base to abstract a proton from the nucleophilic water. At low concentrations of water (i.e., <1 M) in acetonitrile the solvent deuterium isotope effect drops to 1.17 and the proton inventory is consistent with a transition-state structure involving a single nucleophilic water molecule.

Our recent studies[2,3] on the pH-independent, water-catalyzed hydrolysis of the reactive acyl compounds 1-acetylimidazolium ion (**1a**) and 1-acetyl-3-methylimidazolium ion (**1b**) have shown that the rate-limiting transition-state structure involves a water dimer (**2**) with one water molecule serving as a nucleophile and the other as a general base. The studies on the hydrolysis of **1a** in acetonitrile and water mixtures have revealed that a quite drastic alteration in environmental conditions has little or no influence on the structure of the transition state **2**.[4]



1a R:H
1b R:CH$_3$



2

Table I. First-Order Rate Constants for the Hydrolysis of 1-Acetyl-3-methylimidazolium Ion as a Function of Water Concentration (<1 M) in Acetonitrile at 50.00 ± 0.05 °C

[H$_2$O], M	10^5k_1,[a] s^{-1}	10^4k_2,[b] M^{-1} s^{-1}
0.327	4.88 ± 0.06	1.49 ± 0.02
0.516	7.51 ± 0.31	1.46 ± 0.06
0.654	9.47 ± 0.08	1.45 ± 0.01
0.794	11.62 ± 0.39	1.46 ± 0.05
0.892	12.77 ± 0.25	1.43 ± 0.03

[a] Error limits are standard deviations. [b] These values were obtained by dividing the first-order rate constant by the water concentration.

Table II. First-Order Rate Constants for the Hydrolysis of 1-Acetyl-3-methylimidazolium Ion as a Function of Water Concentration (>2 M) in Acetonitrile at 48.50 ± 0.05 °C

[H$_2$O], M	10^4k_1,[a] s^{-1}	[H$_2$O], M	10^4k_1,[a] s^{-1}
2.04	1.78 ± 0.02	4.07	6.28 ± 0.03
2.64	2.75 ± 0.01	4.75	8.32 ± 0.02
3.06	3.55 ± 0.03	5.65	11.74 ± 0.17

[a] Error limits are standard deviations.

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