Catalytic Proton Bridge in Acetylimidazolium Ion Hydrolysis Implicated by a Proton Inventory

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Received January 25, 1977

The origin of the solvent isotope effect, $k_{H_2O}/k_{D_2O} = 2.58$, for the water-promoted hydrolysis of acetylimidazolium ion has been probed using the proton inventory technique. The proton inventory suggests that the observed effect is comprised of three transition state contributions. A chemical model is proposed for the hydrolysis transition state which contains a catalytic proton bridge between the reorganizing substrate and a water molecule serving as a general base catalyst.

The importance of acyl transfer and hydrolysis reactions in biochemical systems is well established and these reactions have been the object of considerable study.[2] Special emphasis has been placed on determining the importance and mechanisms of proton transfer in a variety of systems which can serve as models for the myriad biological systems.[3] In order to fully delineate the mechanism of biological acyl transfer reactions it is necessary to understand their nonbiological analogues in extreme detail. As part of a continuing effort to develop sophisticated techniques for the elucidation of such biochemical mechanisms we have applied the proton inventory technique to such a system.

The reactions of acetylimidazole have been studied under a variety of conditions by Jencks and co-workers.[4] We report here a study of the pH-independent water-promoted hydrolysis of acetylimidazolium ion in mixtures of protium oxide and deuterium oxide. Such a study constitutes a proton inventory and allows us to suggest likely roles for the water molecules in the transition state for this hydrolysis reaction.

Experimental Section

Materials. Acetylimidazole was prepared by the method of Boyer[5] and had mp 99–100 °C (lit.[5] mp 101.5–102.5 °C). Acetonitrile (Fisher reagent grade) was stirred over calcium hydride overnight, distilled from calcium hydride through a 30-cm fractionating column packed with glass helices, and stored under a nitrogen atmosphere. Deuterium oxide (99.8 atom % deuterium, Aldrich) was purified by distillation in an all-glass apparatus before use. Water was glass distilled before use. Sodium chloride (Fisher Certified) and concentrated hydrochloric acid (Mallinckrodt analytical reagent) were used as obtained.

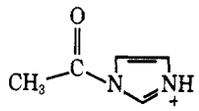
Kinetics. The hydrolysis of acetylimidazolium ion was monitored by following the decrease in absorbance at 245 nm using a Cary 118C UV–vis spectrophotometer equipped with a constant temperature cell compartment and cell holder to control the temperature at 25.00 ± 0.05 °C.

Reactions were initiated by injecting 50 μl of a stock solution which was 6×10^{-3} M acetylimidazole in acetonitrile into 3.00 ml of the appropriate HCl, DCl, or HCl–DCl solution. Stock 0.02 N HCl and DCl solutions in H_2O and D_2O, respectively, were prepared from concentrated hydrochloric acid. The ionic strength was maintained at 0.20 with sodium chloride. The amount of protium introduced into the 0.02 M DCl solution in D_2O in this manner was determined on a sample of the pure DCl–D_2O solution by Mr. Josef Nemeth.[6] This factor has been considered in the data analysis. Reactions in H_2O–D_2O mixtures were done using appropriate volumes of the HCl–H_2O and DCl–D_2O stock solutions.

Reactions were followed to greater than 80% completion and infinity absorbances were taken at 10 half-lives. The pH(D) of the reaction solutions was measured at the completion of each run using a Leeds and Northrup Model 7413 expanded scale pH meter equipped with a combination electrode. First-order rate constants were determined using a nonlinear least-squares computer program which calculates first-order rate constants from given time and absorbance values. These constants were confirmed by plots of log $(A_t - A_\infty)$ vs. time.

Results and Discussion

The hydrolysis of acetylimidazole was studied in 0.02 N HCl (DCl) and 0.02 N HCl–DCl mixtures at 25.00 ± 0.05 °C. Jencks and Carriuolo had previously shown that the hydrolysis of acetylimidazole exhibits a plateau in the pH–rate profile below about pH 3.[4a] This suggested that the reaction observed below pH 3 is the simple water-catalyzed hydrolysis of acetylimidazolium ion (1).



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The presence of this plateau in the pH–rate profile allowed the present study of the hydrolysis of 1 to be conducted in isotopic solvent mixtures in the absence of buffer components in order to characterize the role of the water molecules in this hydrolysis reaction.

Table I and Figure 1 show the dependence of the observed first-order rate constants on the isotopic composition of the solvent. Also included in Table I are calculated values of the observed rate constants based on a chemical model discussed below. The solid line drawn through the data points in Figure 1 is based on this chemical model.

The observed rate constants in the "pure" isotopic solvents are in good agreement with those reported in the literature.[4a] The solvent isotope effect, k_{H_2O}/k_{D_2O}, was determined to be 2.58 in this study, which is also in excellent agreement with the value of 2.5 in the literature.[4a] The nonlinear dependence of the rate constant on the atom fraction of deuterium in the solvent is obvious from Figure 1. The implications of this result will be discussed below.

Proton Inventory Background. A study of the dependence of a reaction rate constant on the atom fraction of deuterium in the solvent has been appropriately termed a proton inventory.[7] The magnitude of a measured solvent isotope effect allows speculation about its origin and the role of proton transfers, general and specific acid–base catalysts, nucleophilic catalysts, and solvent molecules in the reaction mechanism. The proton inventory allows one to suggest chemical models consistent with the observed solvent isotope effect. An analysis of each model allows us to specify the sites expected to contribute to the isotope effect. The magnitude of the contribution of each site to the observed effect can also be specified within reasonable limits for each model considered.

The theory of the proton inventory is well documented in the literature and is presented only in limited detail here.[8] Several recently published inventories serve to further illustrate its potential.[9] The observed reaction rate constant, k_n,

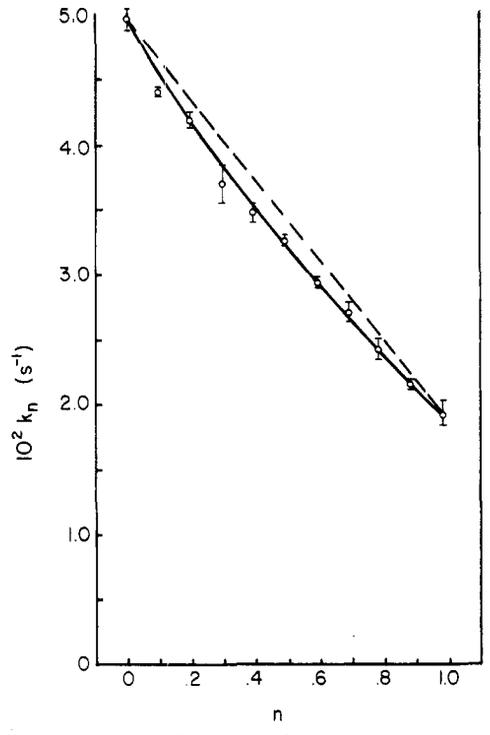


Figure 1. Dependence of the observed first-order rate constants for the hydrolysis of acetylimidazolium ion on the atom fraction of deuterium in the solvent. The data are taken from Table I. The solid line is calculated for the chemical model in (3) using $\phi_a^* = 0.55$ and $\phi_b^* = 0.83$. The dashed line is included to emphasize the nonlinear nature of the data.

in an H_2O–D_2O mixture is related to the rate constant in pure H_2O, k_0, by

$$k_n = k_0 \prod_i^{TS} (1 - n + n\phi_i^*) \Big/ \prod_j^{RS} (1 - n + n\phi_j) \qquad (1)$$

The rate constant in a given H_2O–D_2O mixture, specified by the atom fraction of deuterium n, is seen to depend on the ratio of i transition state (TS) terms to j reactant state (RS) terms. Each exchangeable isotopic transition state site i will be characterized by an isotopic fractionation factor ϕ_i^* and each exchangeable isotopic reactant state site j will be characterized by a similar factor ϕ_j. These isotopic fractionation factors are defined by eq 2. They express the deuterium

$$\phi_k = ([D]/[H])_k/([D]/[H])_{solvent} \qquad (2)$$

preference for the site in question relative to the deuterium preference for an average solvent site. Fractionation factors less than unity imply a greater preference for deuterium in the solvent than in the site in question (i.e., a greater preference for protium in the site in question). Since protium, the lighter isotope, tends to accumulate where the binding is weaker the site in question must contain the isotopic atom in a binding potential weaker than that in the bulk solvent. The inverse of this argument can be used to show that fractionation factors greater than unity are associated with binding potentials tighter than those in the bulk solvent for the isotopic atoms in question.

The curvature exhibited by a plot of k_n vs. n depends upon the magnitude of the observed solvent isotope effect and the number of transition state and reactant state contributors to the measured effect. It can be seen in eq 1 that only sites which change fractionation factor on going from the reactant state to the transition state will be important in determining the solvent isotope effect. A site whose fractionation factor remains the same in the reactant state and transition state will contribute equal terms to the denominator and numerator of eq 1 and they will cancel one another.

Table I. First-Order Rate Constants for the Hydrolysis of Acetylimidazolium Ion in Mixtures of 0.02 N HCl–H_2O and 0.02 N DCl–D_2O at 25.00 ± 0.05 °C [a]

Atom fraction of deuterium (n)	No. of runs [b]	$10^5 k_n$, s^{-1}	$10^5 k_n$ calcd, [c] s^{-1}
0.000	8	4966 ± 81 [d]	4966
0.098	3	4413 ± 32	4590
0.196	4	4193 ± 62	4213
0.294	3	3710 ± 149	3889
0.392	3	3497 ± 55	3563
0.490	3	3267 ± 47	3253
0.587	3	2937 ± 42	2961
0.685	3	2707 ± 67	2682
0.783	4	2430 ± 77	2417
0.881	2	2165 ± 35	2167
0.979 [e]	5	1926 ± 100	1930

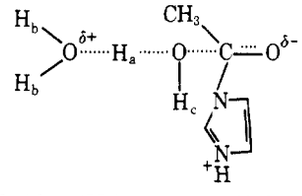
[a] Ionic strength was maintained at 0.20 with NaCl. [b] Combined runs from two independent experiments conducted by different workers. [c] Calculated based on the model in (3) using $\phi_a^* = 0.55$ and $\phi_b^* = 0.83$. [d] Error limits are standard deviations. [e] Atom fraction of deuterium in "100%" 0.02 N DCl–D_2O as determined by Mr. Josef Nemeth.[6]

For a reaction in which all the exchangeable reactant state sites have $\phi = 1.0$ the denominator of eq 1 becomes unity. This is frequently the case when all of the isotopic reactants are solvent molecules whose exchangeable isotopic sites have fractionation factors of unity by the definition of eq 2. In these cases we need only consider the transition state contributions to the solvent isotope effect.

Contributions by more than one proton in the transition state will result in curvature in plots of k_n vs. n except under highly unlikely circumstances involving cancellations of large numbers of transition state and reactant sites.[10] The analysis of a nonlinear proton inventory is illustrated in the discussion of acetylimidazolium ion hydrolysis.

Fitting a Chemical Model to the Observed Proton Inventory for the Hydrolysis of 1. The analysis of the proton inventory for the hydrolysis of 1 is simplified somewhat since the denominator of eq 1 can be neglected. It then becomes necessary to formulate a reasonable chemical model for the transition state and compare the predicted proton inventory for this model with the experimental inventory. Reasonable models, in this case, must involve multiple protons in order to account for the curvature in the plot of k_n vs. n.

A transition state model based on information gleaned from the study of general base catalysis of this reaction[4] and utilizing the concept of a "catalytic proton bridge" of Schowen and co-workers[7,11] is shown in (3).



(3)

The proton bridge (H_a) serves to link a water molecule acting as a general base catalyst to the reorganizing substrate function. This model is consistent with the observation of general base catalysis in the hydrolysis of acetylimidazolium ion and the fact that the "water point" falls on the Brønsted line ($\beta = 0.34$).[4c]

The model in (3) has four isotopically exchangeable protons which could contribute to the observed solvent isotope effect. The proton (H_a) being transferred to the water molecule

acting as the general base should contribute a primary solvent isotope effect to the overall effect. Such protons frequently exhibit fractionation factors of about 0.5 which corresponds to an isotope effect contribution of $k_{H_2O}/k_{D_2O} \sim 2$ for this "in-flight" proton.[8c] The proton H_c should have a fractionation factor near unity and will thus contribute nothing to the observed effect. The two H_b protons will contribute secondary isotope effects and would exhibit fractionation factors of 0.69 each if H_a was fully transferred to generate a fully developed hydronium ion.[8c] If H_a is not transferred to any extent at all in the transition state then the fractionation factors for each H_b would be unity. We have thus established reasonable limits for the fractionation factors associated with the two H_b protons.

A quantitative estimate of the fractionation factors for each H_b can be made using an extension of the Brønsted hypothesis as illustrated in eq 4.[8e]

$$\phi_b^{TS} = (\phi_b^{RS})^{1-\beta}(\phi_b^{PS})^{\beta} \quad (4)$$

The transition state fractionation factor for each H_b is determined by the extent of development of hydronium ion character by the general base water molecule in the transition state. This is correlated with the Brønsted β value. Substituting unity for the reactant state fractionation factor for H_b (ϕ_b^{RS}), 0.69 for the product state (i.e., a full hydronium ion) fractionation factor for H_b (ϕ_b^{PS}) and the observed Brønsted β of 0.34 into eq 4 we calculate a transition state fractionation factor (ϕ_b^{TS}) of 0.88. Substitution of this value for ϕ_b^*, values of the observed rate constant in protium oxide and deuterium oxide, and the value of n which corresponds to "pure" deuterium oxide (0.979 in this case) into eq 5 allows us to calculate a value of ϕ_a^* consistent with the observed effect. This gives $\phi_a^* = 0.53$ and would correspond to a primary isotope effect contribution of $k_{H_2O}/k_{D_2O} = 1.89$ for H_a. Equation 5 is a three-proton version of the generalized form in eq 1 based on a solvent isotope effect attributable to only three transition state contributions as depicted in (3). Substitution of various values of n into eq 5 allows one to calculate a theoretical proton inventory for this model.

$$k_n = k_0(1 - n + n\phi_a^*)(1 - n + n\phi_b^*)^2 \quad (5)$$

It was necessary to slightly alter the values of ϕ_a^* and ϕ_b^* in order to generate a model-based proton inventory consistent with the experimental inventory. The values of k_n calculated using this refined model ($\phi_a^* = 0.55$ and $\phi_b^* = 0.83$) are included in Table I for comparison with the experimental values. The solid line of Figure 1 is based on this model and accurately describes the nonlinear nature of the experimental inventory.

Conclusion

The transition state model in (3) having $\phi_a^* = 0.55$ and $\phi_b^* = 0.83$ describes the observed proton inventory sufficiently well to suggest that the transition state for the water-catalyzed hydrolysis of acetylimidazolium ion does indeed involve a catalytic proton bridge between a water molecule acting as a general base and the reorganizing substrate. This is highly similar to the transition state structure suggested earlier by Wolfenden and Jencks.[4b] Other models consistent with the proton inventory alone could be derived but the model of eq 3 is chemically consistent with the observed general base catalysis and Brønsted β value.

The results of this study show that the proton inventory technique can be used to confirm mechanisms suggested by classical Brønsted data and give a more detailed picture of the transition state structure for the reaction. The implications for mechanistic studies of enzymatic systems are equally important. Such systems do not lend themselves to the buffer catalysis studies required for making a Brønsted plot but are susceptible to study using the proton inventory technique. The excellent agreement illustrated for the two techniques in this system emphasizes the potential of this mechanistic probe in such biological studies.

The observation of a proton bridge serves to illustrate the potential importance of such catalytic mechanisms in enzyme catalysis. Recent proton inventories of several enzymatic systems thought to utilize charge-relay type mechanisms have implicated the involvement of such bridges in some cases. However, one cannot conclude that all simple "water reactions" will employ such proton bridges and exhibit nonlinear proton inventories. A recent inventory of the water-promoted hydrolysis of bis(4-nitrophenyl) carbonate gave a linear dependence of k_n on n at 50 °C.[9d] Clearly more work is needed in this area before general trends and factors controlling the shapes of proton inventories will be obvious.

Acknowledgments. Grateful acknowledgment is made to the Robert A. Welch Foundation and the Texas A&M College of Science Organized Research Fund for support of this work.

Registry No.—**1**, 31346-45-9.

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