able to promote a chain reaction promoting reduction of TI in the dark until termination via eq 22 ends the process. Evidence for such a process is found by following the bleaching of TI absorption at 546 nm by flash photolysis. In contrast to the experiments with TI–TEA, the bleaching is much slower and persists up to 2 ms following the flash. However, in contrast to TI–TEA no ESR signal for TIH· is observed when solutions of TI and BNAH are irradiated; in fact steady photolysis in the ESR spectrometer reveals no readily detectable radical signals. However, photochemical ESR experiments with TI–BNAH in acetonitrile to which the spin trap nitrosodurene has been added lead to a complex spectrum which can be shown to consist of the spectrum of TIH· superimposed on a strong six-line spectrum attributable to the adduct of BNA· to ND. Addition of ND also quenches appreciably the conversion of TI to TIH_2 by BNAH.

The much lower quantum efficiency for reduction of NDI by BNAH can probably be attributed to the lack of production of kinetically "free" radical by a step analogous to eq 19. Thus for NDI with BNAH all reaction probably procedes via a singlet radical pair which efficiently decays to starting material or forms products but in either case does not survive long enough to yield radicals. The contrast in reaction efficiencies between TI and NDI is especially remarkable in that the two dyes both have apparently the same potential for the chain reaction (e.g., similar quenching efficiencies, excited state energies, and redox potentials) but the evident lack of leakage from quenched singlets to long-lived triplet radical pairs for NDI prevents this path from occurring.

The overall patterns of reactivity observed in this study with potentially electron accepting indigo dyes and donors such as TEA and BNAH are ones which should be fairly general. The individual reactivity of both the electron acceptors and donors insofar as the various electron and proton transfer steps are concerned is not unusual and indeed several similar reaction sequences have been observed with other chromophores. What is more unique about the present results is the coupled sequence of electron–proton–electron transfer resulting from encounter of an excited acceptor with a donor. The rapid occurrence of such a process under conditions not especially sensitive to free radical scavengers or other reagents could offer an attractive route for accomplishing chemically useful conversions. Although relatively low quantum efficiencies are associated with the "concerted" process in the present cases, it is reasonable to anticipate that future investigations can lead to reactve combinations that can result in net energy storage with relatively high efficiencies.

Acknowledgment. We are grateful to the U.S. Department of Energy (contract DEAC02-84ER13151) for support of this research.

Registry No. 1, 522-75-8; **2**, 33934-64-4; **3**, 2533-00-8; TEA, 121-44-8; 1-benzyl-1,4-dihydronicotinamide, 952-92-1.

Sequential General Base–Acid Catalysis in the Hydrolysis of RNA by Imidazole[1]

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Contribution from the Department of Chemistry, Columbia University, New York, New York 10027. *Received October 4, 1985*

Abstract: The cleavage of poly-U by imidazole buffer was studied by using a recently described kinetic assay technique. A bell-shaped profile is seen for rate vs. buffer protonation state, but the reaction is kinetically first order in buffer. It is proposed that a sequential base–acid mechanism is involved: the base catalyzes formation of a 5-coordinate phosphorus isomer of the starting material by transferring a proton from nucleophilic hydroxyl to equatorial oxygen, and the acid then catalyzes the forward decomposition of that intermediate to form the products. Some kinetically equivalent alternative mechanisms have been excluded. The proton transfer catalysis mechanism is suggested for the enzyme ribonuclease as well.

The hydrolysis of RNA by bovine pancreatic ribonuclease involves catalysis by an imidazole group acting as a general base and an imidazolium group acting as a general acid.[3] Additional binding interactions are present, and a lysine cation may act as a third catalytic group by coordinating to the phosphate anion of RNA. The hydrolytic sequence involves two steps. In the first one there is a transesterification in which the 2′-hydroxyl group of a ribose unit attacks the phosphate ester, with the formation of a 2′-3′ cyclic phosphate and the cleavage of the RNA chain. In a second step the enzyme catalyzes the hydrolysis of this cyclic phosphate intermediate.

Both steps apparently have similar mechanisms, involving the general base catalysis by imidazole and general acid catalysis by imidazolium ion. A principal piece of evidence for this is the bell-shaped pH vs. rate profile, with a rate maximum when the base group is unprotonated and the acid group carries its proton. However, there is also evidence about these mechanisms from X-ray studies of complexes of the enzyme with substrate and with transition-state analogues.[4]

We have described the bifunctional catalysis of a phosphate ester hydrolysis related to that performed by the enzyme ribonuclease.[5] We mounted two imidazole groups on the primary face of β-cyclodextrin and showed that this catalyst would hydrolyze a cyclic phosphate ester of 4-*tert*-butylcatechol. The selectivity of the hydrolysis could be understood in terms of the geometry expected for general base delivery of a water molecule to the phosphate group.[6] The pH vs. rate profile for this reaction also was bell-shaped as in the enzyme, indicating that the catalysis

(1) Support of this work by the NIH is gratefully acknowledged.

(2) Awardee of Le Pret d'Honneur and N.S.E.R.C. Canada postdoctoral fellowships.

(3) (a) Blackburn, P.; Moore, S. *The Enzymes*; Academic Press: New York, 1982; Vol. 15, Chapter 12, pp 317–433. (b) Richards, F. M.; Wycoff, H. W. Ibid., 1971, Vol. 4, Chapter 24, pp 647–806. Cf. also: Deakyne, C. A.; Allen, L. C. *J. Am. Chem. Soc.* **1979**, *101*, 3951.

(4) Gilbert, W.; Petsko, G. *Biochemistry*, submitted for publication. See also: Wlodawer, M.; Miller, M.; Sjolin, L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3628–3631.

(5) Breslow, R.; Doherty, J.; Guillot, G.; Lipsey, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227.

(6) Cf. also: Breslow, R.; Bovy, P.; Lipsey Hersh, C. *J. Am. Chem. Soc.* **1980**, *102*, 2115. There is indeed a change in selectivity in the direction reported, but the 50:1 product ratio is an overestimate, because of an analytical error.

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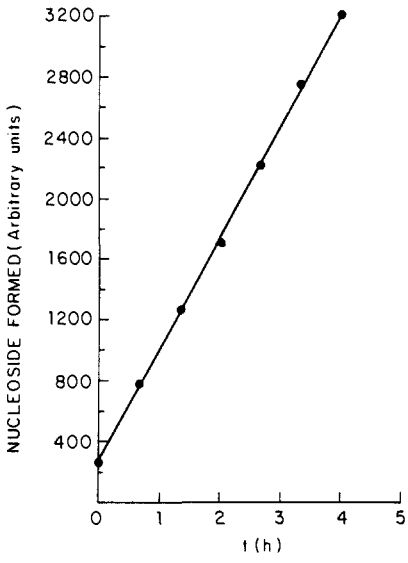

Figure 1. A typical kinetic run. Note that the cleavage is carried out to only a few percent, so the concentration of the substrate is essentially unchanged. Thus is is not necessary to use a logarithmic plot.

was indeed bifunctional and required both the base and the acid to be present for optimal rate. Both in the case of ribonuclease itself and in the case of this model system there is little evidence about the detailed pathway by which the bifunctional catalysis occurs.

Many processes relating to ribonuclease chemistry have been examined with simple catalyst species in solution.[7] Usher has examined the hydrolysis of the phenyl phosphate ester of 3,4-dihydroxytetrahydrofuran.[8] The rate as a function of pH furnished some imformation about the mechanism involved. In particular, he found kinetic evidence for an intermediate, related to our findings on RNA to be discussed. Closely related studies were done by Satoh and Inoue on phenyl phosphate esters of guanosine.[9] The use of these phenyl esters was related to kinetic convenience, since defined spectroscopic changes occurred when the phenoxide groups were liberated. However, it would clearly be desirable to examine the kinetics of cleavage of RNA itself.

We have recently described kinetic methods that permit the study of RNA cleavage.[10] Specifically, our techniques give a defined signal every time a cut is made in poly(uridylic acid) (poly-U). This signal reflects the liberation of a free 5′-OH group and does not change if the 2,3-cyclic phosphate product (cf. Figure 4) is hydrolyzed further. With this method it is possible to examine the catalysis of this cleavage by simple or complex enzyme mimics. Our first study, reported here, concerns the catalysis of RNA cleavage by imidazole buffers. The results show a dependence of rate on concentrations and on protonation states of the buffers that permit us to deduce the operation of a sequential catalytic mechanism. This mechanism may well reflect the one that operates in the enzyme ribonuclease itself. In subsequent publications we will report the use of this kinetic method to examine the catalysis of RNA cleavage by polyfunctional compounds with attached binding groups.

Results and Discussion

The Kinetic Assay Method. As we have described elsewhere,[10] treatment of RNA or its fragments with phosphodiesterase I, a venom exonuclease, cleaves the 3′-phosphate bond. Thus poly-U is converted to U-5′-phosphate. However, in any place in which ribonuclease or a ribonuclease mimic has cleaved the RNA, the 5′-phosphate bond has already been broken; the action of the venom exonuclease leads to the production of a nucleoside, in our case uridine. This can be quantitatively detected by high-pressure liquid chromatography on a very small scale. We have shown that the assay is reliable and leads to good reproducible kinetic results when we examine simple base hydrolysis of poly-U, for instance.[10] This technique cannot detect *neighboring* cuts in RNA, so we run the kinetic assays to only about 5% or so of total RNA cleavage. Under these conditions it has been shown[10] that we obtain a good linear response reflecting the number of cuts that have been made in the poly-U.

The details of the kinetic procedure are reported in the Experimental Section. In Figure 1 we show the results of a typical kinetic run. As can be seen, with a given concentration of imidazole buffer we obtain good pseudo-first-order rate behavior.

Over the concentration range used (0.125–2.0 M) the pseudo-first-order rate constants were also first order in the total concentration of the imidazole buffer, as is shown in Figure 2. There is no sign of a deviation from linearity in buffer concentration, although as Figure 2 shows the slope of the line is definitely a function of the degree of protonation of the buffer. This point is clear in Figure 3, a plot of the pseudo-first-order rate constant

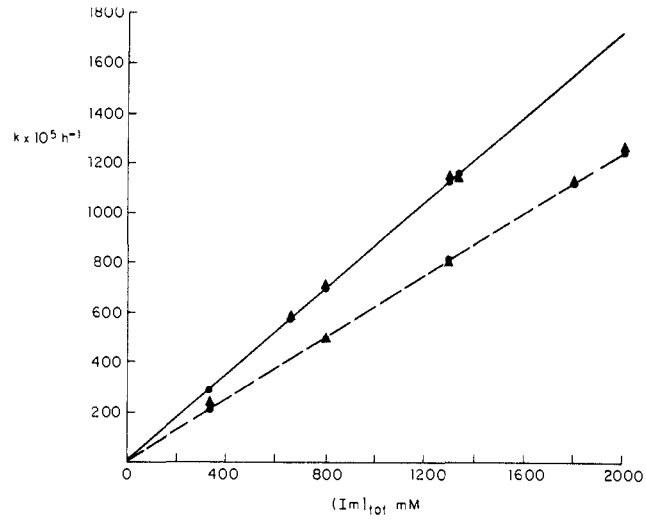

Figure 2. A plot of the observed pseudo-first-order rate constant for poly-U cleavage as a function of imidazole buffer concentration: (●) calculated by our kinetic treatment; (▲) observed. The solid line is for $ImH^+/Im = 0.25$ and the dashed line for $ImH^+/Im = 0.75$.

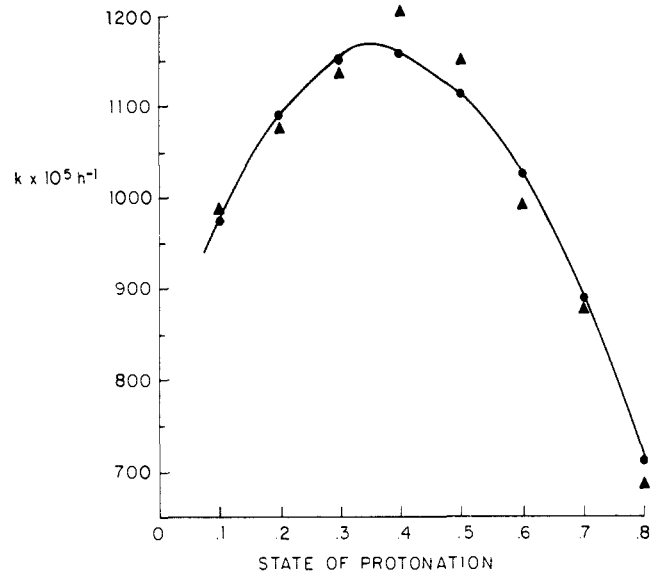

Figure 3. The buffer-catalyzed rate constant at 1.3 M buffer vs. the fraction of buffer in the ImH^+ form. A plot of the data of section C, Table I. (●) Calculated by our kinetic treatment; (▲) observed.

(7) (a) van Aken, D.; Paulissen, L. M. C.; Buck, H. M. *J. Am. Chem. Soc.* **1981**, *46*, 3189. (b) Abell, K. W. Y.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1171. (c) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 510. (d) Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 1521. (e) Eftink, M. R.; Biltonen, R. L. *Biochemistry* **1983**, *22*, 5134.

(8) Oakenfull, D. G.; Richardson, D. I.; Usher, D. A. *J. Am. Chem.* **1967**, *89*, 5491.

(9) Satoh, K.; Inoue, Y. *Chem. Lett.* **1972**, 1097–1100.

(10) Corcoran, R.; Labelle, M.; Czarnik, A. W.; Breslow, R. *Anal. Biochem.* **1985**, *144*, 563–568.

vs. the protonation state for a 1.3 M total imidazole concentration.

The data in Figure 3 can be considered to be related to a typical bell-shaped pH vs. rate constant curve, although it is obvious in this curve that the buffer-catalyzed term is greater on the basic side with imidazole than on the acid side with imidazolium. In the data in Figure 3 we have subtracted any buffer-independent terms, by extrapolation to zero buffer concentration. Thus the data of Figure 3 suggest catalysis by both imidazole and imidazolium ion, as in ribonuclease and in the enzyme model described earlier. The optimum rate occurs at about 35% protonation, not 50%. However, any interpretation involving simultaneous catalysis by both acid and base runs into the difficulty that Figure 2 shows a clean *first-order* dependence of the rate constants on the buffer concentration. Thus the kinetic term that contributes to the maximum rate in Figure 3 cannot involve a product of imidazole and imidazolium concentrations, since no square term in buffer concentration is detectable in Figure 2.

One simple way around this problem would be to invoke complexing, so that doubling the concentration of the buffer only doubled the concentration of the catalytically important aggregate. However, we can detect no complexing of imidazole with imidazolium ion in this concentration range. For instance, the NMR signals for the protons on C-4 and C-5 of imidazole/imidazolium come in precisely the expected average positions of those measured for solutions containing entirely imidazole or entirely imidazolium ion. Even more convincing, cryoscopic measurements (see Experimental Section) of the freezing point of a 1 to 1 solution of imidazole and imidazolium chloride at a total concentration of 1 M and also at a total concentration of 0.5 M showed, within experimental error, exactly the expected freezing point depressions for independent particles. Thus by both NMR and cryoscopic measurements no significant complexing of the two buffer forms can be detected; certainly there is not enough to explain the kinetic observations, which would require essentially complete complexing.

A second possibility is that an imidazolium ion complexes with poly-U. However, we have titrated imidazole potentiometrically in the presence and absence of poly-U and do not detect an appreciable change in the pK_a. Although one might have expected a cation to bind to the polyanion of poly-U, the poly-U is of course prepared as a potassium salt. Only if imidazolium ion were strongly bound to poly-U in *preference* to potassium ion could we invoke complexing that might hide a higher order kinetic term. Apparently no such strong complexing occurs.

Since the reconciliation of the behavior in Figures 2 and 3 cannot be achieved by invoking some special complexing interactions, it must be accounted for in terms of the operative mechanisms. A bell-shaped pH vs. rate profile can of course indicate simultaneous acid–base catalysis of a reaction, but it also can indicate a change in the rate-determining step.[11] For example, if the first step of the reaction requires base and the second step requires acid then if we start on the basic side the addition of acid can increase the rate as the second step speeds up, but eventually the second step becomes fast enough that now the first step is rate-limiting. Under these circumstances the further addition of acid will decrease the rate, because the slow first step becomes slower as the base component is removed. This is the behavior that we believe we have detected in our kinetic studies.

Assuming a simple scheme with intermediate B

$$A \underset{\text{cat 1}}{\overset{\text{cat 1}}{\rightleftharpoons}} B \xrightarrow{\text{cat 2}} C \qquad (1)$$

a standard steady state (of intermediate B) treatment yields

$$\text{rate} = \frac{k_1 k_2 [1][2]}{k_2[2] + k_{-1}[1]}\,[\text{substrate}] \qquad (2)$$

Of course eq 2 does not fully describe the behavior of the data in Figure 3. It correctly accommodates the maximum rate when both catalyst 1 and catalyst 2 are present but predicts a rate of zero when one of them is missing. This can be handled by adding terms reflecting several possibilities.

(1) There is an additional pathway from A to C catalyzed only by cat 1 and another with cat 2. This would allow non-zero rates at the extremes.

(2) The path from A to B, and B to C, can also be catalyzed by water, but more poorly than by cat 1 and cat 2. This would add more kinetic terms resembling those of eq 2 and allow non-zero termini with an intermediate maximum as in Figure 3.

(3) Both the A to B and B to C steps can be catalyzed by either cat 1 or cat 2, but with a reversed kinetic preference. Thus again the rates at the extremes of Figure 3 would be non-zero, but less than optimal.

Since a detailed treatment of these cases does not permit us to select among them, we will not present the mathematics here. However, in all cases the conclusion is similar: the path represented by eq 1 is the best, and other lesser paths play a role chiefly when one of the two catalysts is missing.

We have treated all these possible paths in detail by Simplex optimization[12] of the relevant rate constants. In Figure 3 we present a calculated curve for alternative 1, in which cat 1 is imidazole, cat 2 is imidazolium, and there are independent paths with k'_{Im} and k'_{ImH+} leading directly from A to C. As can be seen, with appropriate rate constants this fits the data reasonably well for $k_1 = 1.7298 \times 10^{-2}$ h^{-1}, $k'_{Im} = 6.180 \times 10^{-3}$ h^{-1}, and $k'_{ImH} = 1.766 \times 10^{-3}$ h^{-1}. Only the ratio of k_2/k_{-1} can be derived: 1.158. For example, $k_2 = 9.867 \times 10^{-4}$ h^{-1} and $k_{-1} = 8.524 \times 10^{-4}$ h^{-1}. We also show the calculated points on Figure 2 using these constants.

Treatment of the other alternatives gives similar fits to the experimental data, but with different values for the rate constants. Although we have plotted the simplest version, we think alternatives 2 or 3 are more likely: the same pathway is always used, but with alternative catalysts.

The Sequential Base–Acid Mechanism. In a two-step mechanism (eq 1) there must be a reaction intermediate. The only sensible intermediate on the way between RNA and the 2′-3′ cyclic phosphate is a five-coordinate phosphorus species, in which the nucleophile has attacked the phosphorus but the leaving group has not yet been lost. It is easy to see that this intermediate must be an isomer of the starting material (with or without extra water molecules) and that it must therefore also be a monoanion. If there were one less proton on it, so it were a dianion, then the first step of the two-step sequence would involve imidazole attacking the substrate to produce an imidazolium salt of the dianion. Now this imidazolium cation could catalyze the reversal of the first step or the forward reaction in the second step, but a change in concentration of imidazolium ion would not change the partitioning between forward and reverse reaction of the intermediate. Doubling the concentration of the imidazolium ion would cut the concentration of the intermediate in half at the same time that it would double the rate of forward reaction of the intermediate. Thus if the intermediate is missing a proton relative to the starting material one would expect no rate increase from increasing the concentration of imidazolium ion, contrary to what is observed. If by contrast the first step is catalyzed by imidazolium ion, and it produces an intermediate with one extra proton relative to the starting material, it is easy to see that the catalysis by imidazole would disappear. Thus we can specify not only that this reaction goes through a five-coordinate phosphorus intermediate but that that intermediate must be the monoanion **3**.

Of course it has been commonly believed that phosphate-substitution reactions of this sort proceed through a five-coordinate phosphorus intermediate,[13] but we believe that this is the first kinetic evidence for RNA requiring an intermediate.[14] Nonkinetic evidence also exists, including our finding that the hydrolysis of UpU (diuridyl 3′,5″ phosphate) by OH^- leads to the formation of the 2′,5″ isomer accompanying hydrolysis. Such a migration

(11) For a classic early example, cf. the pH–rate profile for oxime formation elucidated by Jencks: Jencks, W. P. *J. Am. Chem. Soc.* **1959**, *81*, 475.

(12) Ryan, P. B.; Barr, R. L.; Todd, H. D. *Anal. Chem.* **1980**, *52*, 1460.

(13) Cf.: Westheimer, F. *Chem. Rev.* **1964**, *64*, 317.

(14) Usher, in ref 8, presents related evidence for a model phosphate ester. However, his proposed mechanism is rather different from ours, as was the kinetic behavior of his system.

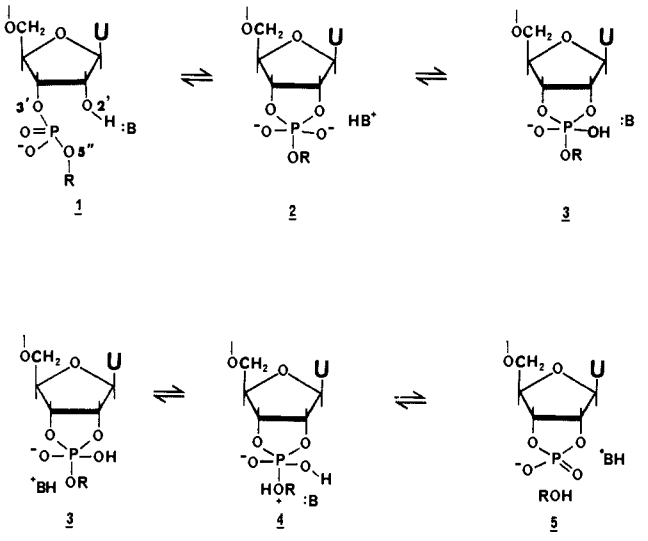

Figure 4. Our preferred mechanism. See text for discussion.

must occur through a five-coordinate phosphorus intermediate with pseudorotation.[13] In our present study we suggest a mechanism (Figure 4) in which imidazole reversibly converts poly-U to the intermediate **5** and imidazolium ion catalyzes the conversion of **5** to the cleaved product.

The mechanism seen here (Figure 4) is quite curious. In the first step nucleophilic attack is catalyzed when the imidazole removes a proton from the hydroxyl of **1** at C-2, the resulting nucleophilic oxygen adds to phosphorus, and the temporarily formed imidazolium ion puts a proton back on one of the phosphate oxygens of **2** to generate intermediate **3**. In the reversal of this reaction the same sequence runs backward, and loss of the C-2 oxygen occurs by deprotonation of intermediate **3**, and eventual protonation of the leaving oxygen. However, in the forward direction intermediate **3** loses the 5′ oxygen of the next nucleotide by a process preferentially catalyzed by imidazolium ion, not by imidazole. In the imidazolium ion catalysis a proton is presumably being added to the leaving group first and then at some later stage a proton is being removed from the phosphate oxygens so that the final product monoanion **5** is formed.

Why is it that loss of the C-5″ oxygen from intermediate **3** occurs by a prior protonation of the leaving group oxygen, while loss of the C-2′ oxygen from **3** occurs by a prior deprotonation of an oxygen on the phosphorus? If in the alternative slower path the loss of the 5″ oxygen can also occur with imidazole catalysis, and thus with a prior deprotonation, why is it that the prior protonation mechanism is not the best for the loss of C-2′ oxygen? It is hard to see a good reason for this mechanistic distinction, although there is of course a big difference in the loss of these two oxygens. Loss of the 5″ oxygen is driven by the entropy gain on fragmentation, while loss of the 2′ oxygen is driven by the enthalpy gain when a strained ring is opened. Perhaps this enthalpy gain is enough to permit the extrusion of an unprotonated oxyanion, while the loss of the 5″ oxygen is better if the prior protonation or at least hydrogen bonding assists it to leave.

The well-known limitations of kinetic evidence are present here. Alternative mechanistic sequences are also possible, most obviously the mechanistic sequence in which the roles of the acid and the base are reversed. That is, a mechanism in which the formation of intermediate **3** is catalyzed by imidazolium ion, and its forward decomposition is catalyzed by imidazole, cannot be excluded on the basis of our kinetic data.

Significance of Our Mechanism for the Enzyme Ribonuclease. We believe that the mechanism that operates here may well be similar to that used by ribonuclease itself. It has generally been assumed that the basic imidazole of the enzyme (of histidine-12) acts to remove the proton from the attacking C-2′ hydroxyl group, but we suggest that it then transfers that proton to one of the new equatorial phosphate oxygens, so that the intermediate formed is still a phosphate monoanion paired with the lysine cation. Then it has also been generally assumed that the imidazolium ion of the enzyme (of histidine-119) acts to put a proton on the leaving 5″ oxygen atom, but we suggest that after it does this the now deprotonated imidazolium ion reaches up and removes the proton from one of the phosphate equatorial oxygens so as to form a product monoanion. Thus we suggest that in the enzyme the imidazole groups serve not simply to remove or add protons but instead to move them from one part of the substrate to another.

This is related to the function of the imidazole in chymotrypsin.[15] The imidazole of histidine-59 removes a proton from serine but then adds that proton to the leaving nitrogen atom. In the case of ribonuclease the geometric requirements at a five-coordinate phosphorus are such that it is not possible for a single imidazole group to shuttle protons between the attacking and leaving atoms, since they occupy opposite apical positions. However, transferring protons between these apical positions and an equatorial oxygen of the intermediate would be stereochemically possible and catalytically useful. Of course obtaining the kind of kinetic evidence that could establish such a mechanism is even more difficult in the enzymatic case than in the simple case we have described.

Table I. Cleavage of poly-U by Imidazole Buffer[a]

	state of protonation[b]	$[Im]_{tot}$,[c] mM	$10^5 k_{obsd}$,[d] h^{-1}	$10^5(k_{obsd} - k_0)$,[e] h^{-1}
A	0.25	125	145	110
		333	306	275
		667	611	580
		1333	1162	1131
		2000	1734	1699
	0.375	125	150	96
		500	516	462
		1200	1096	1042
		2000	1812	1758
	0.625	125	159	108
		500	436	385
		1200	966	911
		2000	1618	1567
	0.75	125	160	72
		333	300	205
		667	503	408
		1333	913	818
		2000	1324	1488
B	0.25	800	726	701
		1300	1171	1146
	0.375	800	730	699
		1300	1181	1150
	0.50	800	723	674
		1300	1177	1128
	0.625	800	651	591
		1300	1051	955
	0.75	800	551	492
		1300	858	799
C	0.1	1300	1129	1115
	0.2	1300	1237	1214
	0.3	1300	1315	1286
	0.4	1300	1397	1360
	0.5	1300	1343	1298
	0.6	1300	1171	1118
	0.7	1300	1050	990
	0.8	1300	842	774

[a] At 80.0 ± 0.2 °C in H_2O. [b] Fraction of the imidazole buffer added as imidazole hydrochloride. [c] Final concentration of the imidazole buffer. [d] The pseudo-first-order rate constant for poly-U cleavage. [e] The buffer-catalyzed component, after subtracting the extrapolated rate constant at zero buffer concentration.

Experimental Section

Materials and Kinetic Method. We have described our kinetic assay method in detail elsewhere.[10] As before, we used polyuridylic acid potassium salt of average molecular weight >100 000 purchased from P-L Biochemicals. Phosphodiesterase I was obtained from Sigma. Great care was taken to avoid contamination by foreign ribonuclease.

(15) Cf.: Zubay, G. *Biochemistry*; Addision-Wesley Publishing Co.: Reading, MA, 1983; Chapter 4.

The kinetic assay procedure was as described[10] except for the following points: (a) the capillary tubes used as kinetics vessels were not silylated before use; (b) buffer A used in the phosphodiesterase digestion was made 50 mM in $MgCl_2$, not 18.8 mM; (c) the pH electrode monitored pH adjustment of the reaction mixture and pH correction solutions required by the assay[10] were avoided by adjusting the state of protonation of the catalyst for the reaction mixture, and by making the correction solution from the calculated amounts of acid or base required to titrate 1 μL of each reaction mixture back to pH 7.00.

Imidazole, half-protonated imidazole, and imidazole hydrochloride were all prepared as 6 M stock solutions from recrystallized imidazole and freshly titrated concentrated HCl and used to make up the kinetic reaction mixtures.

Cleavage of poly-U by Imidazole Buffers. The data are listed in sections A, B, and C of Table I. For section A, 140 μL of solution was prepared 62.3 mM in poly-U, 1.03 mM in potassium *p*-nitrobenzenesulfonate hplc standard, and imidazole catalyst as listed. Each solution was divided into 10 melting point capillary tubes which were sealed and incubated at 80.0 ± 0.2 °C. For section B, 95 μL of solution was divided among 8 tubes. For section C, only 7 points per run were done.

Cryoscopy of the Buffer. A solution 0.99 M in total imidazole, half each imidazole and imidazole hydrochloride in H_2O, gave a melting point depression of 2.501 °C for H_2O. This corresponds to 1.358 molal independent solute particles (calcd 1.484). At half the concentration it gave a 1.302 °C depression, corresponding to 0.70 molal particles (calcd 0.74). For calibration, 0.1 N NaOH in H_2O gave ΔT 0.395 °C, corresponding to 0.212 molal particles (calcd 0.020).

Registry No. Im, 288-32-4; ImCl, 1467-16-9; poly-U, 27416-86-0; ribonuclease, 9001-99-4.

Transition Structures for C- and O-Alkylation of Acetaldehyde Enolate. Stereoelectronic Effects and C/O Alkylation Ratios

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Abstract: The transition structures for the gas-phase C- and O-alkylations of acetaldehyde enolate with methyl fluoride have been located with ab initio RHF calculations and the 3-21G basis set. Single point calculations have been carried out with the 6-31G* basis set. The activation energy for O-alkylation is lower than that for C-alkylation, even though the latter is favored thermodynamically. These results parallel those found for the reactions of Me^- and HO^- with MeF and arise from the lower intrinsic barrier for reactions of oxygen-centered nucleophiles (or leaving groups) than carbon-centered nucleophiles in S_N2 reactions. The geometries of the transition structures indicate that stereoelectronic factors favor product-like conformations, even for relatively early transition states.

Alkylations of enolates are synthetically valuable and mechanistically intriguing processes.[1,2] Many hypotheses have been advanced about the factors which control the ratios of C- and O-alkylation[1-5] and the stereoelectronic factors controlling the direction of attack of the alkylating agent on the enolate.[6-9] We report insights into both of these phenomena obtained from ab initio transition structures for the C- and O-alkylation of acetaldehyde enolate by methyl fluoride.

Results and Discussion

Transition structures were located with ab initio molecular orbital calculations using the 3-21G basis set.[10] Anion–neutral complexes formed between the two reactants and the two products were also optimized at this level, since these anion–molecule complexes are known to be intermediates in gas-phase S_N2[11-13] and enolate reactions.[12,14] Reactions of the methyl anion and the hydroxide ion with methyl fluoride were also studied, in order to compare the C- and O-alkylation of enolates to the analogous reactions of localized species. Single point calculations on reactants and transition structures were also carried out at the 6-31G* level with use of the 3-21G optimized geometries. These calculations are designated as 6-31G*//3-21G.[15]

The relative energies of the stationary points on these surfaces are summarized in Figure 1, while salient geometrical features are summarized in Figures 2 and 3.[16-17] In the gas phase, as in solution, the C-alkylated product is more stable than the O-alkylated. At the 6-31G*//3-21G level, propionaldehyde is 23 kcal/mol more stable than methyl vinyl ether, whereas this difference is 18 kcal/mol from Benson's group equivalents.[18] Ac-

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