The Reaction of Thiols with Acetylimidazole. Evidence for Independent Reaction Pathways[1]

William P. Jencks* and Karin Salvesen

Contribution No. 762 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received July 23, 1970*

Abstract: The reactions of acetylimidazole with weakly acidic thiols undergo a change in rate-determining step with increasing imidazole buffer concentration. The imidazole-catalyzed step is assigned to the breakdown of a tetrahedral addition intermediate and the other step to attack of thiol anion on free acetylimidazole. However, the concurrent, uncatalyzed reaction of acetylimidazolium ion with thiol anion does *not* undergo this change in rate-determining step. The phosphate-catalyzed reaction also does not undergo a change in rate-determining step and phosphate can bring about a rate increase under conditions in which imidazole brings about no further rate increase. It is suggested that these results may be explained in terms of bimolecular reactions in aqueous solution that proceed by independent, concurrent pathways; *i.e.*, the intermediates or transition states need not be at equilibrium with respect to transport processes.

Chemists interested in reactions in solution have generally avoided the question of what happens on the way to the transition state. In a sense this is necessary and proper because transition state theory assumes that reactants and catalysts are at some sort of equilibrium with respect to the transition state, so that the question of how this quasi-equilibrium state is reached may be avoided. However, it is often further assumed that for moderately slow reactions these species are also at equilibrium with respect to transport processes and solvent rearrangement; *i.e.*, that the observed reaction rate reflects a chemical process taking place in the transition state and that the free energy of the transition state reflects the free energy of activation for this chemical process, including any association or solvent rearrangement that has occurred in rapid, prior-equilibrium steps to provide the lowest possible free energy. This further assumption is, of course, not necessary since transport processes themselves can be described by transition state theory. It should, therefore, be possible to set some limits on what happens as the transition state is approached with respect to what is going on in the rest of the solution. Evidence has been reported recently suggesting that a diffusion-controlled proton transfer step is rate determining for a relatively slow acyl transfer reaction in aqueous solution.[2] We report here evidence which suggests that different catalyzed and uncatalyzed reactions of thiols with acetylimidazole may proceed by concurrent, independent paths which are not at equilibrium with each other with respect to transport processes.

Experimental Section

The experimental procedure has been described previously.[3-5] Organic reagents were redistilled or recrystallized; thiols were distilled under nitrogen.

Solutions of 0.5–2.0 $\times$ 10^{-2} M acetylimidazole were prepared shortly before use in 10^{-3} M imidazole. A stock solution was stored at 0° and aliquots were brought to room temperature shortly before use. Solutions of thiols were prepared shortly before use in water which had been flushed with argon. Solutions of mercaptoacetic acid were neutralized to pH 6–7 in the presence of 10^{-3} M ethylenediaminetetraacetic acid and stored under argon in a number of stoppered tubes, each of which was used for several runs. Ethylenediaminetetraacetic acid, 1–4 $\times$ 10^{-4} M, was added to reaction mixtures to retard heavy metal ion catalyzed oxidation of thiols. Experiments with mercaptoacetate were particularly susceptible to interference by oxidation, as evidenced by an increase in the base line absorption, and the cuvettes in which these reactions were carried out were sometimes flushed with argon before the addition of acetylimidazole.

Pseudo-first-order rate constants in the presence of a large excess of thiol were evaluated spectrophotometrically with a Gilford recording spectrophotometer by following the disappearance of acetylimidazole (2–7 $\times$ 10^{-4} M) at 260–270 nm. The acetylimidazole was added to the reaction mixture immediately after the addition of thiol. Reactions with the volatile ethanethiol were carried out by adding a cold solution of thiol to a cuvette which was stoppered and equilibrated at 25° before the addition of acetylimidazole. Individual stoppered tubes containing the stock solution of ethanethiol were not used for more than two runs. The concentration of ethanethiol was determined by reaction with Ellman's reagent.[6] Reactions of phenyl acetate were followed at 275 or 282 nm. Most runs were carried out in duplicate or triplicate. Pseudo-first-order rate constants were calculated from half-times, which were read directly from the spectrophotometer tracings over several different portions of the reaction. The ionic strength was generally maintained constant with tetramethylammonium chloride for experiments with amine buffers and with potassium chloride for experiments with phosphate buffers. A number of comparisons showed that the nature or concentration of salt had little effect on the rate constants over the range of concentration examined. The pH was determined in each reaction mixture after completion of the kinetic determination.

The experimental conditions and observed catalytic constants, based upon total buffer concentration, for the reactions of acetylimidazole with a series of thiols are summarized in Table I. Rate constants were corrected for hydrolysis or the direct reaction of acetylimidazole with the buffer if necessary, but such corrections were usually negligible.

Product Analysis. Acetylimidazole, 0.002 M, was found to give yields of thiol ester, measured as active ester reacting with neutral hydroxylamine,[7] of 96–103% after reaction with 0.01 M mercaptoacetate in 0.025–0.3 M imidazole buffers, 50% base, and 0.1 M *N*-methylmorpholine buffer, 30% base. Acetylimidazole, 0.01 M, was found to give a product with an absorption maximum typical

(1) Publication No. 762 of the Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154. Supported by grants from the National Science Foundation (GB 5648) and the National Institute of Child Health and Human Development of the National Institutes of Health (HD-1247).

(2) R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, 91, 2358 (1969).

(3) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, 234, 1272, 1280 (1959).

(4) D. G. Oakenfull and W. P. Jencks, *J. Amer. Chem. Soc.*, 93, 178 (1971).

(5) D. G. Oakenfull, K. Salvesen, and W. P. Jencks, *ibid.*, 93, 188 (1971).

(6) G. L. Ellmann, *Arch. Biochem. Biophys.*, 82, 70 (1959).

(7) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, 159, 21 (1945).

Table I. Buffer Catalysis of Reactions of Thiols with Acetylimidazole at 25°

Thiol	Buffer	% base	pH	Concn range, M	No. of points	Ionic strength	Salt	k_{cat},[a] M^{-2} min^{-1}
Mercaptoacetate[b]	Imidazole	5	5.8	0.04–0.3	8	0.2	Me_4NCl	[c]
		20	6.5	0.04–0.17	8	0.2	Me_4NCl	[c]
		50	7.1	0.001–0.4	41	0.2	Me_4NCl	[c]
		80	7.8	0.01–0.30	22	0.2	Me_4NCl	[c]
		90	8.1	0.01–0.30	15	0.2	Me_4NCl	[c]
	Methylmorpholine	30	7.4	0.02–0.58	14	0.6	Me_4NCl	890[c,d]
		95	8.8	0.01–0.30	7	0.6	Me_4NCl	1300
	Tris(hydroxymethyl)-aminomethane	10	7.3	0.025–0.19	4	1.0	Me_4NCl	640[e]
		20	7.7	0.025–0.21	8	1.0	Me_4NCl	760[e]
		50	8.3	0.025–0.42	8	1.0	Me_4NCl	1240[e]
	Triethylenediamine	16	8.4	0.025–0.42	10	1.0	Me_4NCl	1450
		30	8.7	0.025–0.42	9	1.0	Me_4NCl	2400
	Chloroquinuclidine	15	8.3	0.04–0.17	9	1.0	Me_4NCl	1040
		30	8.7	0.04–0.17	8	1.0	Me_4NCl	1760
	Quinuclidinol	10	8.8	0.02–0.21	9	0.2	Me_4NCl	670[f]
		20	9.2	0.02–0.21	8	0.2	Me_4NCl	1560[f]
Mercaptoethanol[g]	Imidazole	1[h]	5.2	0–0.30	6	1.0	KCl	[i]
		5	5.8	0.01–0.29	12	0.2	Me_4NCl	[c, i]
		8	6.1	0.01–0.29	6	1.0	KCl	[c, i]
		15	6.5	0.01–0.29	12	1.0	KCl	[c, i]
		15	6.4	0.01–0.29	9	0.3	Me_4NCl	[c, i]
		30	6.8	0.01–0.21	7	0.2	Me_4NCl	[c, i]
		50	7.2	0.001–0.58	17	0.3	Me_4NCl	[c, i]
		65	7.4	0.02–0.58	15	0.3	Me_4NCl	[c, i]
		90	8.1	0.04–0.29	10	0.3	Me_4NCl	[c, i]
		90	8.1	0.01–0.21	7	0.2	Me_4NCl	[c, i]
	Methylmorpholine	5	6.4	0.02–0.58	12	0.6	Me_4NCl	[c, j]
		20	7.1	0.02–0.58	12	0.6	Me_4NCl	[c, j]
		40	7.5	0.01–0.29	7	0.6	Me_4NCl	880
		60	7.9	0.01–0.25	7	0.6	Me_4NCl	1150
		89	8.6	0.01–0.25	7	0.6	Me_4NCl	1300
	Phosphate	30	6.1	0.01–0.29	13	1.0	KCl	310
		30	6.1	0.01–0.16	5	1.0	KCl	310
		50	6.5	0.02–0.58	10	1.0	KCl	490
		50	6.5	0.02–0.25	7	1.0	KCl	520
	Acetate	30	4.2	0.05–0.40	6	1.0	KCl	$\leq$20
		80	5.2	0.05–0.40	6	1.0	KCl	$\leq$20
		99[k]	6.7	0.1–0.3	3	1.0	KCl	$\leq$30
Methyl mercapto-acetate[l]	Imidazole	20	6.5	0.04–0.17	9	0.2	Me_4NCl	2900
		50	7.1	0.04–0.17	10	0.2	Me_4NCl	4900
		50	7.1	0.015–0.34	9	0.2	Me_4NCl	4100
		80	7.7	0.04–0.17	8	0.2	Me_4NCl	5700
	Phosphate	50	6.5	0.01–0.29	9	1.0	KCl	6000

[a] Based on total buffer concentration. Corrected for buffer catalysis of hydrolysis, if significant. [b] 0.016 M. [c] Nonlinear in buffer concentration. [d] Initial slope. [e] Corrected by 6–15% for the reaction of Tris with acetylimidazole. [f] Corrected by 35–40% for the reaction of quinuclidinol with acetylimidazole. [g] 0.009 or 0.018 M. [h] In 0.05 M acetate buffer. [i] See Figure 1. [j] See Figure 3. [k] In 0.02 M phosphate buffer. [l] 0.0018 M.

of thiol esters at 231 nm in 0.01 M hydrochloric acid after reaction with 0.02 M mercaptoethanol in the absence and in the presence of 0.025 and 0.125 M imidazole buffers, 50% base; the yields were 92–94% based on extinction coefficients of 3000 and 5200 for acetylimidazole[8] and *S*-acetylmercaptopropanol,[9] respectively. Rapid acidification of several reaction mixtures at the end of the reaction revealed no hydrolysis of residual acetylimidazole; *i.e.*, the measured reactions had proceeded to completion rather than to give an equilibrium mixture of thiol ester and acetylimidazole.

Results

Catalysis of the reaction of acetylimidazole with mercaptoethanol by imidazole gives a nonlinear increase in rate with increasing buffer concentration; at high buffer concentration and at low pH the rates become independent of imidazole concentration (Figure 1). The leveling off of the rate becomes less marked and the rates eventually approach linearity with respect to imidazole concentration with increasing pH. The rate constants are slightly higher at an ionic strength of 1.0, maintained with potassium chloride (open circles), than at ionic strength 0.2–0.3, maintained with tetramethylammonium chloride (closed symbols). The limiting rate constants at high buffer concentration (obtained from the reciprocals of the ordinate intercepts of plots of $1/(k_{obsd} - k_0)$ against 1/[buffer], where k_{obsd} is the observed second-order rate constant and k_0 is the rate constant at zero buffer concentration) are proportional to hydroxide ion activity (Figure 2). The third-order rate constant for this limiting reaction is 3.6×10^9 M^{-2} min^{-1}. The rate constants also increase nonlinearly with respect to methylmorpholine buffer concentration at low pH values, although methylmorpholine is a less effective catalyst and the leveling off is less marked than with imidazole buffers (Figure 3). Because of the smaller curvature, extrapolations to infinite buffer concentration are less accurate for the methylmorpholine buffers, but within experimental

(8) E. R. Stadtman in "Mechanism of Enzyme Action," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1954, p 581.

(9) W. P. Jencks, S. Cordes, and J. Carriuolo, *J. Biol. Chem.*, **235**, 3608 (1960).

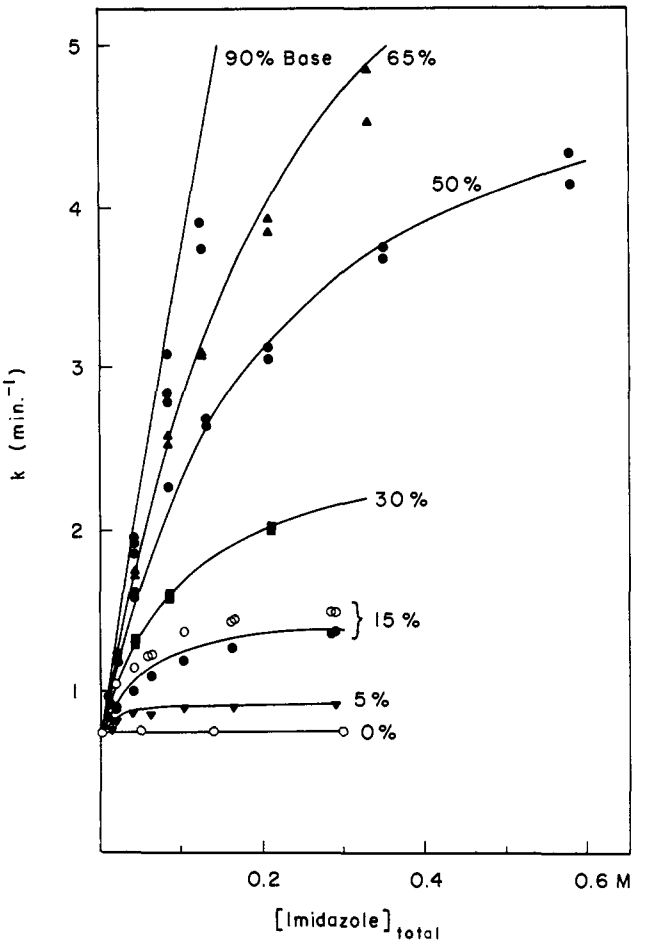


Figure 1. Catalysis by imidazole buffers at the indicated fractions of free base of the reaction of acetylimidazole with 0.009 M mercaptoethanol at 25°. Ionic strength maintained at 0.2–0.3 with tetramethylammonium chloride (closed symbols) or at 1.0 with potassium chloride (open circles). The solid lines were calculated from eq 4.

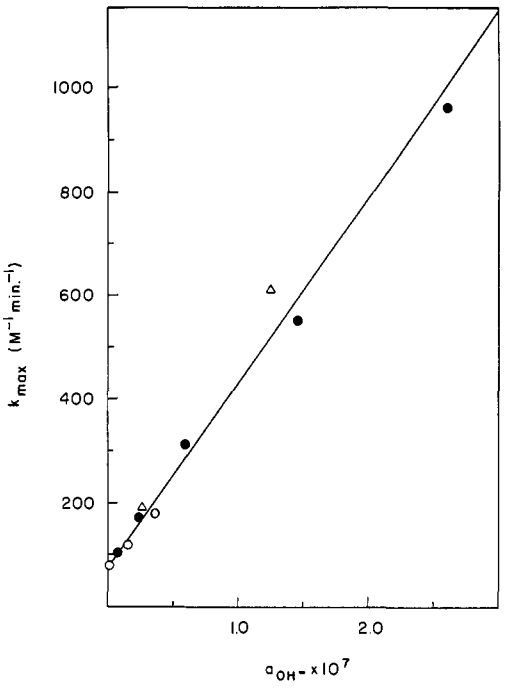


Figure 2. The dependence on hydroxide ion activity of the limiting rate constants at high buffer concentrations for the reaction of acetylimidazole with mercaptoethanol in the presence of imidazole (circles) or methylmorpholine (triangles) buffers at 25°.

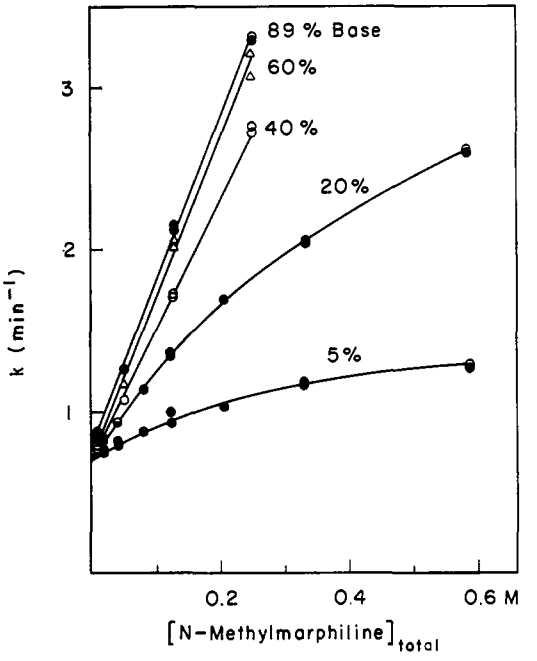


Figure 3. Catalysis by methylmorpholine buffers of the reaction of acetylimidazole with 0.009 M mercaptoethanol over the pH range 6.4–8.6 at 25°. The ionic strength was maintained at 0.6 with tetramethylammonium chloride.

error the limiting rates at high methylmorpholine buffer concentrations are the same as at high imidazole concentrations at the same pH (Figure 2, triangles).

In contrast to the tertiary amine buffers, catalysis by phosphate buffers shows little or no leveling off and, although phosphate is a less effective catalyst than imidazole at low buffer concentrations, the observed rate constants at higher buffer concentrations are well above the limiting rates observed in imidazole buffers at the same pH (Figure 4). Furthermore, phosphate is still an effective catalyst when added to reaction mixtures containing imidazole buffers at a concentration high enough to give the limiting rate for imidazole at a given pH value (Figure 4); *i.e.*, phosphate catalysis is not subject to the same rate limitation as imidazole catalysis in either the presence or the absence of imidazole buffer. Although the existence of the catalysis is certain, the catalytic constant for phosphate appears to be significantly decreased in the presence of imidazole, possibly because of complexation between phosphate and a component of the imidazole buffer.

The rate constants for the reaction with mercaptoethanol extrapolated to zero concentration of imidazole and methylmorpholine buffers (Figures 1 and 3) are independent of pH in the range pH 5.9–8.6; the second-order rate constant k_0 (eq 1) is 80 M^{-1} min^{-1} at ionic

$$v = k_0[\mathrm{AcIm}][\mathrm{RSH}] = k_0'[\mathrm{AcImH^+}][\mathrm{RS^-}] \quad (1)$$

strength 0.2–0.3 (tetramethylammonium chloride) and 90 M^{-1} min^{-1} at ionic strength 1.0 (potassium chloride). The same rate constants were obtained in acetate buffers at pH 4.2 and 5.2 (corrected for the protonation of acetylimidazole to acetylimidazolium ion, pK = 3.86[4]) and in the absence of buffer at a pH of approxi-

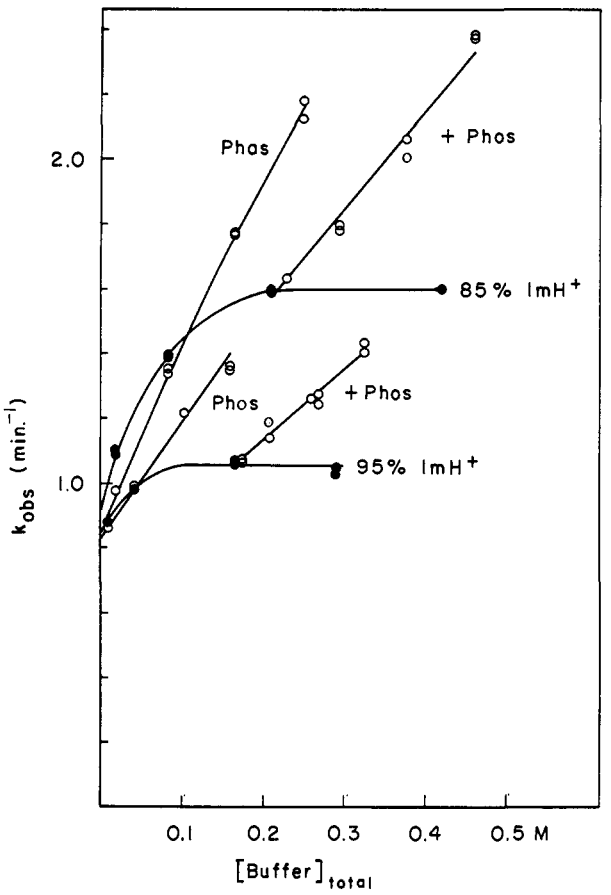


Figure 4. Catalysis by phosphate, by imidazole, and by mixtures of the two buffers of the reaction of acetylimidazole with 0.009 *M* mercaptoethanol at 25°, ionic strength maintained at 1.0 with potassium chloride.

mately 7.3. There is little or no catalysis of the reaction by acetate buffers, nor by acetate ion in the presence of 0.02 *M* phosphate buffer at pH 6.7 (Table I).

Catalysis of the reaction of acetylimidazole with mercaptoacetate ion by imidazole and methylmorpholine buffers was found to exhibit the same behavior with respect to buffer concentration and pH; the leveling off occurs at slightly lower buffer concentrations and pH values compared to the mercaptoethanol reaction. Catalysis by phosphate buffers is relatively less effective with mercaptoacetate, perhaps because of electrostatic repulsion between the two anions, so that a comparison of the effects of phosphate and amine buffers was not attempted. The reactions with mercaptoacetate are technically more difficult to follow than those with mercaptoethanol, in part because of a relatively high background absorption and oxidation of the thiol, and linear double reciprocal plots of rate against buffer concentration were not always obtained. However, the results of a large number of experiments (Table I) gave a value of $k_0 = 41\ M^{-1}\ \text{min}^{-1}$ (from extrapolation to zero buffer concentration in the pH range 6.4–9.2 and in the absence of buffer, pH approximately 7.3) and a limiting rate constant proportional to hydroxide ion concentration in the presence of high concentrations of imidazole and methylmorpholine buffers of approximately $1.8 \times 10^9\ M^{-2}\ \text{min}^{-1}$ (ionic strength 0.2–1.0, tetramethylammonium chloride). The rate constants were found to increase linearly with thiol concentration up to 0.016 *M* in the presence of 0.02 *M* and 0.29 *M* imidazole buffer, pH 7.1. At lower pH values the observed rate constants increase, presumably because of the appearance of a reaction proportional to the concentration of free mercaptoacetic acid. The rate constant for the reaction of this acid (pK = 3.67) was estimated from experiments at pH 4.6 and 5.5 (extrapolated to zero concentration of acetate buffer) to be approximately 2200 $M^{-1}\ \text{min}^{-1}$, which is similar to the value of 2800 $M^{-1}\ \text{min}^{-1}$ for methyl mercaptoacetate (see below).

Both the basic and acidic species of imidazole and methylmorpholine buffers are active as catalysts (Figures 1 and 3). The rate constants for catalysis by a series of buffers were obtained from runs at high pH values, at which the rates approach linearity with respect to buffer concentration, or from the initial slopes of plots against buffer concentration at lower pH values and are summarized in Table II.

Table II. Catalytic Constants for the Reaction of Acetylimidazole with Thiols at 25°

Thiol	Buffer	pK	Catalytic constant, $M^{-2}\ \text{min}^{-1}$ k_B[a]	k_{BH^+}[b]
Mercapto-ethanol	Imidazole	7.2	3900	1450
	Methylmorpholine	7.7	1400	650[c]
	Phosphate dianion	6.6	1010	
	Acetate	4.6	$\lesssim$30	$\lesssim$30
Mercapto-acetate	Imidazole	7.2	3800[c]	1270[c]
	Methylmorpholine	7.7	1270[c]	730[c]
	Tris(hydroxy-methyl)amino-methane	8.3	2000	480
	Triethylenediamine	9.1	8500 4250[d]	
	3-Chloroquinuclidine	9.04	6400	
	3-Quinuclidinol	9.83	7200	
Methyl mercaptoacetate	Imidazole	7.2	6600	2000

[a] For the basic form of the buffer. [b] For the acid form of the buffer. [c] Approximate value. [d] Statistically corrected.

The reaction of acetylimidazole with ethanethiol (pK = 10.35) in imidazole buffers (Table I) was found to show almost exactly the same behavior as the reaction with mercaptoacetate (pK = 10.24), but detailed kinetic studies were not attempted with this very volatile thiol.

The reaction of acetylimidazole with methyl mercaptoacetate (pK = 7.91) is considerably faster than that with other thiols with a value of $k_0 = 2800\ M^{-1}\ \text{min}^{-1}$. The catalytic constants for imidazole (Table II) do not increase as rapidly as k_0 with increasing acidity of the thiol, so that imidazole catalysis, although definite, is small, with only 15–30% increases in the observed rate constants at the highest buffer concentrations. There is no indication of a leveling off of the rate at high buffer concentrations.

There are several lines of evidence which show that the leveling of the rate constants for the reactions of acetylimidazole with basic thiols with increasing concentrations of imidazole and methylmorpholine buffers at low pH values is not caused by complexation of a component of the buffer with one of the reactants.

(a) There is little or no such leveling in the imidazole-catalyzed reactions of acetylimidazole with a number of other nucleophilic reagents[3-5] including methyl mercaptoacetate.

(b) Complexation of thiol with imidazolium cation, which would be required to explain the leveling off at low pH values, would cause an inhibition of the base line rate at very low pH values, but no such inhibition is observed (Figure 1).

(c) The fact that phosphate is still an effective catalyst under conditions in which the imidazole-catalyzed reaction has leveled off (Figure 4) means that the reactants are available for the phosphate-catalyzed reaction and are not bound in an unreactive complex.

(d) The pH of a 0.05 *M* imidazole buffer, 30% base, undergoes no significant change in the presence of up to 0.6 *M* added mercaptoethanol (a decrease of 0.03 pH unit was observed at the highest thiol concentration); *i.e.*, there is no complexation of mercaptoethanol with one component of the buffer.

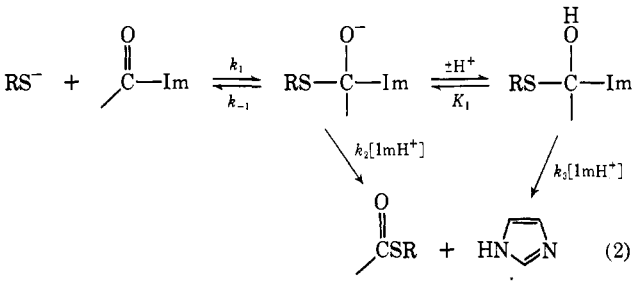
(e) Imidazole and thiol do not form an unreactive complex, because the reaction of mercaptoacetate with phenyl acetate is not inhibited by imidazole buffer (Figure 5). In the course of these experiments it was noted that a significant back-reaction of phenol with acetylimidazole occurs under the conditions ordinarily used for rate measurements and leads to erroneously high observed rate constants; the addition of 0.01 *M* mercaptoacetate to trap the acetylimidazole eliminates this problem.

(f) The observed rate constants increase linearly with increasing thiol concentration at both low and high imidazole concentrations.

Discussion

The change in rate-determining step with increasing buffer concentration in the reaction of acetylimidazole with weakly acidic thiols is strong evidence that there is an intermediate in the reaction, and that either the formation or breakdown (but not both) of this intermediate is catalyzed by the buffer.[10] The pH-dependence of the reaction at limiting imidazole buffer concentrations indicates that the step which is not subject to buffer catalysis occurs at a rate that is proportional to hydroxide ion activity under conditions in which the thiol is predominantly in the nonionized form.

The mechanism of eq 2 for the catalyzed reaction is



(2)

consistent with these results. Buffer catalysis is ascribed to the donation of a proton by a general acid to the leaving imidazole of an anionic or neutral tetrahedral addition intermediate, to account for general base and general acid catalysis, respectively. Evidence

(10) For other examples, see S. Johnson, *Advan. Phys. Org. Chem.*, 5, 237 (1967); W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 10.

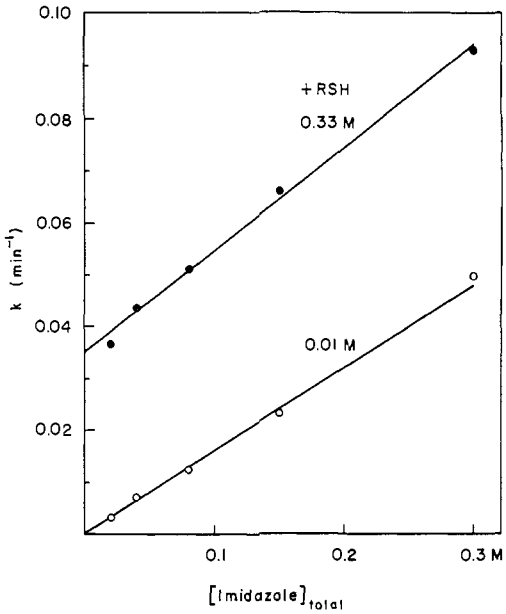


Figure 5. Observed pseudo-first-order rate constants for the disappearance of 0.003 *M* phenyl acetate in the presence of imidazole buffers, 50% base, and the indicated concentrations of mercaptoacetate at 25°, ionic strength maintained at 0.5 with potassium chloride.

has been reported that catalysis of the reactions of acetylimidazole with amines that have a basicity similar to that of the thiol anions considered here involves proton donation to the leaving imidazole, but there is no evidence for a kinetically significant intermediate in the amine reactions.[5] It is reasonable that a change in rate-determining step should occur in the thiol and not the amine reactions, because the high affinity of thiols for the carbonyl carbon atom[11] would be expected to make the thiol anion a poorer leaving group than a protonated amine so that k_{-1} is more likely to be slow enough to be kinetically significant compared to k_2 or k_3 in the thiol than in the amine reactions; *i.e.*, the addition step (if it is a discrete step) is more likely to be at equilibrium in the amine than in the thiol reactions. Similarly, the absence of evidence for a change in rate-determining step in the reaction of methyl mercaptoacetate is consistent with a relatively large value of k_{-1} as a consequence of the low basicity of this thiol. The step which is not subject to buffer catalysis is ascribed to the attack of thiol on free acetylimidazole. It is known that the attack of basic thiol anions on simple carbonyl compounds is not subject to general acid–base catalysis.[12]

The strongest evidence for these assignments comes from the examination of product formation as a function of pH and catalyst concentration from the analogous tetrahedral intermediates that are generated during the hydrolysis of thioimidates.[2,13-15] The low

(11) J. Hine and R. D. Weimar, Jr., *J. Amer. Chem. Soc.*, 87, 3387 (1965), and references therein; E. G. Sander and W. P. Jencks, *ibid.*, 90, 6154 (1968).

(12) G. E. Lienhard and W. P. Jencks, *ibid.*, 88, 3982 (1966).

(13) R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *ibid.*, 81, 5089 (1959); R. B. Martin and A. Parcell, *ibid.*, 83, 4830 (1961); R. B. Martin and R. I. Hedrick, *ibid.*, 84, 106 (1962); R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, 29, 3197 (1964).

(14) R. K. Chaturvedi, A. E. MacMahon, and G. L. Schmir, *J. Amer. Chem. Soc.*, 89, 6984 (1967).

(15) R. K. Chaturvedi and G. L. Schmir, *ibid.*, 91, 737 (1969); G. M. Blackburn, *Chem. Commun.*, 249 (1970).

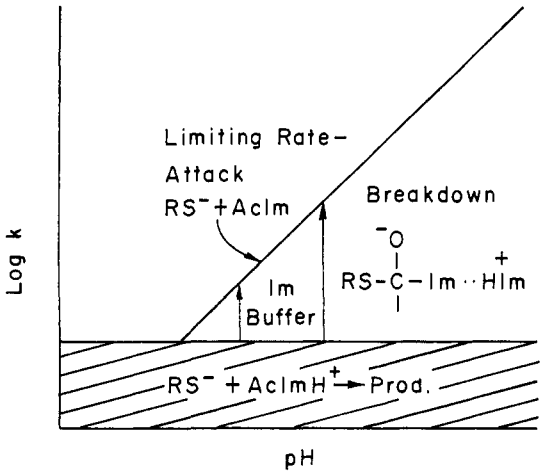


Figure 6. Schematic representation of the dependence on pH of the pathways for the reaction of acetylimidazole with thiols, showing the pH-independent base line reaction with no change in rate-determining step and the catalyzed reaction that proceeds through a tetrahedral intermediate with a change in rate-determining step.

energy steps for the breakdown of such intermediates and, hence, for the overall thiol ester ⇄ amide interconversion are generally the expulsion of thiol anion at high pH and the general acid- or base-catalyzed expulsion of amine at low pH. Hence, the high energy, rate-determining step in the acyl transfer reaction is expected to be thiol attack at low pH and high buffer concentration and amine expulsion at high pH and low buffer concentration. Amine expulsion requires protonation and in some reactions there is evidence that catalysis of this step involves a diffusion-controlled encounter of an acid or base with the intermediate.[2,15] It is not certain whether buffer catalysis of the acetylimidazole reaction involves a chemical process or an encounter-controlled proton transfer in the rate-determining step; there is a small difference in the catalytic effectiveness of amines of differing pK in this reaction (Table II), but not enough to choose definitely between a reaction with a small β value (or large α value) and an encounter-controlled reaction.[16]

The reaction of imidazole with thiol esters, an example of thiol ester aminolysis, is the reverse of the reaction of thiols with acetylimidazole and must proceed with the same transition state and mechanism. Evidence has been reported for a change in rate-determining step and an intermediate in thiol ester aminolysis.[2,13,17] The mechanism of oxygen ester aminolysis involves rate-determining amine attack with general base catalysis of the removal of a proton from the attacking amine at high pH and expulsion of alkoxide ion through an anionic transition state at low pH,[18,19] which is similar to the proposed mechanism (in reverse) for the reaction of thiols with acetylimidazole.

Normally, if a reaction proceeds through an intermediate either the formation or the breakdown of the intermediate is rate determining under a given set of experimental conditions, even if there are several pathways for the formation and breakdown steps. Thus, if a change in rate-determining step occurs this change involves all the pathways for a given step. The remarkable observation in the acetylimidazole reactions is that *the change in rate-determining step that occurs in the imidazole-catalyzed and methylmorpholine-catalyzed reactions does not occur in the uncatalyzed reaction.* The rate of the uncatalyzed reaction is independent of pH over the entire range examined (except for the rate increase at low pH caused by a reaction proportional to free mercaptoacetic acid concentration); there is no indication of the rate decrease with decreasing pH that would be expected if the step proportional to hydroxide ion concentration (*e.g.*, the attack of thiol anion on free acetylimidazole, the k_1 step of eq 2) became rate determining in this reaction as it does in the catalyzed reaction. There cannot be a shift to a rate-determining pH-independent thiol attack step at low pH because then an increase in the base line rate should be observed with increasing pH as the anionic, k_1 pathway becomes significant and provides an additional pathway for the attack step. If pH-independent attack and breakdown steps should have coincidentally similar or identical rate constants, both steps would be partially rate determining at low pH and an increase in the observed rate of twofold or more should be observed with increasing pH as the hydroxide ion catalyzed attack step becomes significant so that the pH-independent attack step is no longer rate determining (*i.e.*, for a two-step reaction with comparable rate constants k_a and k_b, $k_{obsd} = k_a k_b/(k_a + k_b) \approx {}^1/_2 k_b$, whereas if k_a becomes fast, $k_{obsd} = k_b$); furthermore, such a situation requires that there be significant catalysis of the breakdown step by buffers at low pH values if this step is partly rate determining, and this is not observed. We are forced to the conclusion that the uncatalyzed, pH-independent pathway and the buffer-catalyzed pathway proceed independently and concurrently; the intermediate that is formed in the buffer-catalyzed pathway is not at equilibrium with the uncatalyzed reaction pathway.

From the results of studies of other reactions of acetylimidazole with nucleophiles, especially comparisons with reactions of 1-acetyl-3-methylimidazolium ion,[3,5,20] the pH-independent reaction may be formulated as a reaction of thiol anion with the acetylimidazolium cation (eq 3). There is no indication of the

$$RS^- + \underset{\diagup\quad\diagdown}{\overset{O}{\overset{\|}{C}}ImH^+} \underset{k_{-0'}}{\overset{k_{0'}}{\rightleftharpoons}} RS\overset{O}{\overset{\|}{C}} + Im \qquad (3)$$

existence of kinetically significant intermediates along this reaction path. The proton is a stronger acid catalyst than imidazolium ion, so that the protonated imidazole can presumably be expelled easily during or immediately after the attack of thiol with no requirement for further catalysis or a change in rate-determining step.

The proposed mechanism is summarized diagrammatically in Figure 6. The base line reaction of thiol anion

(16) The rate constants required if the k_2 step represents a rate-determining encounter-controlled proton transfer are not unreasonable: if the value of k_2 is taken as 10^8 M^{-1} sec^{-1}, the rate constant for proton transfer from imidazolium ion to imidazole [E. K. Ralph and E. Grunwald, *J. Amer. Chem. Soc.*, **91**, 2429 (1969)], the value of k_{-1} is 1.3×10^7 sec^{-1} and k_1/k_{-1} is 2×10^{-4} M^{-1} for the mercaptoethanol reaction. This value of k_{-1} may be compared to the rate constant of $\geq 2.5 \times 10^8$ sec^{-1} for expulsion of the ethanethiolate anion from the anionic tetrahedral intermediate formed during the hydrolysis of ethyl trifluorothiolacetate (W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, p 522).

(17) T. C. Bruice and L. R. Fedor, *J. Amer. Chem. Soc.*, **86**, 4886 (1964).

(18) B. A. Cunningham and G. L. Schmir, *ibid.*, **89**, 917 (1967).

(19) G. M. Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968).

(20) R. Wolfenden and W. P. Jencks, *ibid.*, **83**, 4390 (1961).

Table III. Summary of Kinetic Constants for the Reactions of Thiols with Acetylimidazole at 25°

Thiol	pK	k_0(RSH, AcIm), M^{-1} min^{-1}	k_0'(RS$^-$, AcImH$^+$) $\times 10^{-7}$, M^{-1} min^{-1}	k(RS$^-$, AcIm, ImH$^+$), M^{-2} min^{-1}	k_1(RS$^-$, AcIm) $\times 10^{-5}$, M^{-1} min^{-1}
Mercaptoacetate	10.24	41	9.9	5.1×10^6	3.2
Ethanethiol	10.35	30[a]	9.3[a]		
Mercaptoethanol	9.61	80	4.4	1.2×10^6	1.4
Methyl mercapto-acetate	7.91	2800	3.2	4.2×10^4	

[a] Approximate value.

with acetylimidazolium cation proceeds independently of pH and of the catalyzed reaction, with no change in rate-determining step. Superimposed on this is a buffer-catalyzed reaction proceeding through an addition intermediate—buffer catalyzes the breakdown of the intermediate and increases the rate until the attack of thiol anion on free acetylimidazole becomes rate determining.

The steady-state treatment gives the rate law of eq 4

$$k_{obsd} = k_0 + \frac{(k_1K_s/k_{-1})(k_2/a_{H^+} + k_3/K_I)[ImH^+]}{1 + k_2[ImH^+]/k_{-1} + k_3[ImH^+]a_{H^+}/k_{-1}K_I} \quad (4)$$

for the concurrent operation of the pathways of eq 2 and 3, with no common intermediate, for the reaction of acetylimidazole with thiols. In eq 4, k_{obsd} (M^{-1} min^{-1}) is based on [RSH] and K_S is the ionization constant of the thiol. The solid lines of Figure 1 were calculated from eq 4 and agree with the experimental data to within a few per cent, well within the experimental error for these pH-dependent reactions. The agreement of the limiting rate constants reached at high concentrations of imidazole and methylmorpholine buffers (Figure 2) provides further support for the mechanism of eq 4; the phosphate-catalyzed reaction is not subject to this same rate limitation (Figure 4).

The effects of thiol structure on the rate constants for these reactions, summarized in Table III, are consistent with these interpretations. The observed, base line rate constants k_0 increase markedly with increasing acidity of the thiol, as expected for a reaction which involves the thiol anion as the reactive species. The rate constants k_0' for reactions of the thiol anion with acetylimidazolium ion show little dependence on pK, with only a threefold increase accompanying a 200-fold increase in basicity. This behavior is similar to that for the reactions of strongly basic oxyanions with acetylimidazolium ion and suggests that the transition state of the base line reaction occurs early along the reaction coordinate with little decrease in the charge on the sulfur atom. In contrast, the rate constant k (RS$^-$, AcIm, ImH$^+$) for the reaction catalyzed by imidazolium ion shows a large dependence on thiol basicity with a 200-fold increase paralleling a corresponding increase in thiol anion basicity. This is the behavior expected if this term represents catalysis of the breakdown of a tetrahedral addition intermediate in which the charge on the attacking sulfur atom has been removed (eq 2, k_2). It is these differing sensitivities of the catalyzed and uncatalyzed reactions to nucleophile basicity that result in the diminished relative importance of the catalyzed compared to the uncatalyzed reaction with increasing thiol acidity; the same trend is well known in the reactions of oxygen esters with amine nucleophiles, for which the relative importance of the catalyzed reaction diminishes with increasing acidity of the leaving alcohol in the series alkyl ester, phenyl acetate, *p*-nitrophenyl acetate.[19,21] Finally, the low sensitivity of the k_1 reaction to thiol basicity is consistent with an early transition state, as expected for the formation of a tetrahedral addition intermediate.

The phosphate-catalyzed reaction also appears to proceed independently of the imidazole-catalyzed reaction pathway. Phosphate, unlike imidazole, can act as a bifunctional acid–base catalyst and it is possible that phosphate may act as a catalyst for both the attack of thiol and the expulsion of imidazole, so that no change in rate-determining step occurs.

Concurrent, Stepwise, Encounter-Limited, and Concerted Reaction Pathways. We can consider three main types of reactions in solution in which the formation of product by a low energy pathway requires a proton transfer, in addition to the process which provides the main energy barrier (such as the formation of a tetrahedral addition intermediate); we will call the latter step the "chemical" process.

(1) In the *normal* mechanism transition states and intermediates are at equilibrium with respect to transport processes. The proton transfer itself may be stepwise or concerted relative to the rest of the reaction and the transition state for breaking and formation of (for example) carbon–nitrogen and carbon–sulfur bonds is reached by a pathway or pathways which need not be specified.

(2) In the *encounter-controlled mechanism* the transition state for a chemical process, such as the formation of a metastable tetrahedral addition intermediate, is followed by a diffusion- or rotation-controlled proton transfer which must occur in order that the overall reaction may take place by a low energy path and which represents the rate-determining step of the overall reaction. There is evidence that this situation holds in certain examples of thiol ester aminolysis and thioimidate breakdown.[2,15]

(3) In the limiting case of the *preassociation mechanism* the catalyst and reactants come together in a fast preliminary step, after which the chemical step(s) occur without further equilibration of intermediates or the transition state with respect to proton transfer to or from other components of the solution. In other words, the concentration of any intermediates on the pathway to the transition state and the rate constants for the proton-transfer steps required for their interconversion are not large enough that a crossover between reaction paths should occur.[22] The preassocia-

(21) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, 82, 675 (1960); T. L. Bruice and M. F. Mayahi, *ibid.*, 82, 3067 (1960).

(22) *Cf.* J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, pp 119–120.

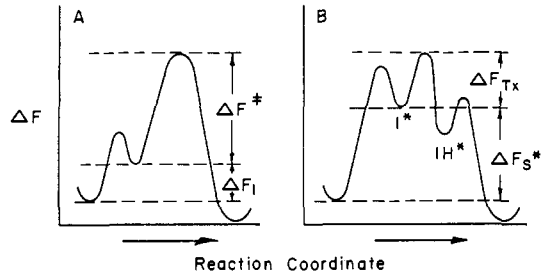


Figure 7. Transition state–reaction coordinate diagrams for (A) reactions in which the reactants undergo preliminary association to form a complex which then undergoes chemical change and (B) reactions in which a chemical change is followed by a rate-determining proton-transfer step.

tion step can give a product ranging in stability from a simple encounter complex, in which the reactants and catalyst are in a solvent "cage," to a reactant with a fully formed chemical bond to the proton (or a completely removed proton). This mechanism is often indistinguishable experimentally from mechanism 1. It may correspond to the "stepwise" mechanism that has been discussed previously for certain acid- or base-catalyzed reactions.[23]

The results described here suggest that the transition states and intermediates in the reactions of acetylimidazole with certain thiols are not at equilibrium with each other with respect to proton transfer, so that the reactions proceed through parallel, concurrent pathways. A possible assignment of these pathways with respect to mechanisms 1–3 is that the base line reaction of thiol anion with acetylimidazolium ion occurs through the preassociation mechanism (3) and the buffer-catalyzed reactions with a kinetically significant addition intermediate proceed through mechanism 1 with classical general acid–base catalysis or mechanism 2 with diffusion-controlled proton transfer from the catalyst. The former reaction may be regarded as a specific acid catalyzed reaction in which imidazole is protonated to give the acetylimidazolium ion (pK = 3.9) in a fast equilibrium step and the acetylimidazolium ion has a sufficiently good leaving group that no change in rate-determining step or further catalysis of the reaction occurs. The buffer-catalyzed reaction pathway involves the addition of thiol anion to free acetylimidazole to give a tetrahedral addition intermediate with a poor leaving group that can break down to expel imidazole only with assistance from catalysis by a proton-donating agent. The solvated proton itself is present in too low a concentration to provide a significant contribution to the observed rate of reaction by this pathway. An argument has been presented previously that the reactions of trifluoroethoxide ion with free acetylimidazole and with acetylimidazolium ion must proceed by separate pathways that are not at equilibrium with each other,[4] and there are other indications or suggestions that tetrahedral addition intermediates may not be at equilibrium with respect to proton transfer.[24]

(23) R. P. Bell and P. Jones, *J. Chem. Soc.*, 88 (1953); C. G. Swain, A. J. DiMilo, and J. P. Cordner, *J. Amer. Chem. Soc.*, 80, 5983 (1958); G. E. Lienhard and F. H. Anderson, *J. Org. Chem.*, 32, 2229 (1967); G. E. Lienhard and T.-C. Wang, *J. Amer. Chem. Soc.*, 91, 1146 (1969).

(24) A. Moffat and H. Hunt, *ibid.*, 81, 2082 (1959); M. L. Bender and H. d'A. Heck, *ibid.*, 89, 1211 (1967); R. Barnett and W. P. Jencks, *J. Org. Chem.*, 34, 2777 (1969); for further references see ref 2.

What determines which of these mechanisms is followed for a given reaction? The situation may be described more clearly with the aid of the transition state diagrams of Figure 7 for some limiting cases. The free energy of activation ΔF_{obsd} for the "normal" mechanism may be arbitrarily divided into the free energy of complex formation, ΔF_1, and the free energy of activation for the chemical process, $\Delta F^{\pm}$ (eq 5 and 6, Figure

$$S + HA \overset{K_1}{\rightleftharpoons} [S\cdots HA] \rightleftharpoons [S^*\cdots H\cdots A]^{\pm} \longrightarrow \text{products} \quad (5)$$

$$\Delta F_{obsd}{}^{\pm} = \Delta F_1 + \Delta F^{\pm} \quad (6)$$

7A).[25] The free energy of complex formation includes the entropy of association of the reactants, amounting to some 2400 cal/mol of free energy for a bimolecular association in aqueous solution based on a molarity standard state. The free energy of activation for the encounter-controlled mechanism may be divided into the free energy for the *equilibrium* interconversion of reactant(s) into an unstable intermediate in which the major chemical process has taken place (*e.g.*, a tetrahedral addition intermediate) and the free energy of activation for the diffusion-controlled encounter of this intermediate with a catalyst, approximately 3000 cal/mol (eq 7 and 8, Figure 7B). The choice between these

$$S \overset{K_{S^*}}{\rightleftharpoons} I^* \overset{HA}{\longrightarrow} I^*H \longrightarrow \text{products} \quad (7)$$

$$\Delta F_{obsd} = \Delta F_{S^*} + \Delta F_{Tx} \quad (8)$$

mechanisms is determined by the sum of the free energies: the former mechanism will be favored when the energetic advantage gained by the presence of the catalyst in the initial chemical process is sufficient to offset a significant part of the entropy loss from the association of reactant(s) and catalyst, whereas the encounter-controlled mechanism will be favored when there is relatively little such gain and the free energy of the equilibrium process ΔF_{S^*} is significantly smaller than $\Delta F^{\pm}$. This situation apparently holds in the intramolecular aminolysis of thiol esters, in which there is little advantage to general acid catalysis of the attack of a strongly basic amine on the thiol ester and the proton transfer steps occur subsequently, in order to prevent regeneration of starting material and permit thiol expulsion.[2]

The preassociation mechanism may be described by the same scheme that has been given for a limiting case of the normal mechanism; it differs from this case only in that intermediates and the transition state are not at equilibrium with respect to transport processes, so that more than a single pathway may proceed concurrently and independently in the same solution at the same time. This mechanism will be favored when chemical intermediates (such as tetrahedral addition compounds) do not exist or are of low stability, so that the likelihood of equilibration with respect to proton transfer is low, and when the initial complex has an increased stability. These conditions may be met in the base line reaction of acetylimidazolium ion with thiol anion since the protonated amide (pK = 3.9) has considerable stability and the protonated imidazole is a good leaving group that may be expelled rapidly from any addition intermediate that is formed before equilibration with respect to proton transfer takes place.

(25) R. A. Marcus, *J. Phys. Chem.*, 72, 891 (1968).

These considerations suggest that the independent or concurrent existence of specific and of general acid or base catalyzed (or "water") reactions may be a special case of the occurrence of independent pathways that are not at equilibrium with respect to transport properties. There are many reactions for which there is no satisfactory explanation of why general acid or base catalysis is not observed or in which solvent catalyzed or stepwise reactions occur concurrently with general acid or base catalyzed reactions and may appear as deviations from Brønsted plots. For example, the reactions that correspond to kinetic general base and general acid catalysis of ketone enolization both involve general base catalyzed removal of a proton from carbon and differ only in that the carbonyl group is protonated in the general acid catalyzed reaction.[23] If the solvated proton can act as a proton donor to the carbonyl group and water can solvate or donate a proton to the same oxygen atom as it develops a negative charge, why is there not more significant catalysis by other proton donors? There may be a meaningful distinction between, for example, a stepwise, specific acid catalyzed mechanism with a rapid equilibrium protonation step and a general acid catalyzed reaction with $\alpha = 1.0$. One would then not necessarily expect to see general acid catalysis even if pathways for (specific) proton catalysis and a solvent reaction occur concurrently. As indicated above, the preassociation mechanism involving specific acid or base catalysis or a "solvent" reaction will be especially favored when the initial equilibrium step is thermodynamically favorable; general acid or base catalysis of reactions in which proton transfer does not provide the main energy barrier is rare or unknown when proton transfer between the catalyst and the appropriate site of the starting material is thermodynamically favorable so that it occurs before the transition state.

The evidence that intermediates and transition states may not be at equilibrium with respect to proton transfer provides support to the suggestion that there may be reactions in which they are not in the most stable equilibrium state with respect to solvent rearrangement.[26] Such cases may be less common than with respect to proton transfer because of the rapid rate of solvent rearrangement[27] and the numerous ways in which a favorable solvent organization might take place along the reaction coordinate.

One of the ways by which enzymes catalyze reactions is undoubtedly by supplying an active site containing appropriate proton-donating and -accepting agents and a microsolvent environment that are already positioned favorably relative to the bound substrate so as to stabilize the transition state of the catalyzed reaction. This avoids the free energy requirement for the proton transfer or solvent rearrangement steps that have been discussed here. The entropy requirements for these processes are supplied by the binding forces of the substrate to the enzyme and by the three-dimensional structure that is built into the active site of the enzyme. There is other evidence that the *rate* of diffusion-controlled proton transfers may be significant in the catalysis of acyl transfer reactions in aqueous solution[2,4,15] and a similar situation has been suggested for certain enzymatic reactions.[28]

Acknowledgment. We are grateful to David Jencks for computations of theoretical curves.

(26) R. P. Bell, *Discuss. Faraday Soc.*, **39**, 16 (1965); C. D. Ritchie, G. A. Skinner, and V. G. Badding, *J. Amer. Chem. Soc.*, **89**, 2063 (1967); J. M. Williams, Jr., and M. M. Kreevoy, *Advan. Phys. Org. Chem.*, 6, 63 (1968).

(27) The dielectric relaxation time of water is 10^{-10}–10^{-11} sec, whereas the time for proton transfer between favorably oriented reactants is probably on the order of 10^{-13} sec; however, the rate of proton transfer in the thermodynamically favorable direction is ordinarily limited by the rate of diffusion or rotation of the reactants and with a second-order rate constant of 10^{-9}–10^{-10} M^{-1} sec^{-1} will be relatively slow in dilute solution; it will be still slower in the thermodynamically unfavorable direction [M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 3, 1 (1964)].

(28) L. Parker and J. H. Wang, *J. Biol. Chem.*, **243**, 3729 (1968).