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The Reaction of Amino Alcohols with Active Esters
II. Catalysis of the Acylation Step by Metal Ions

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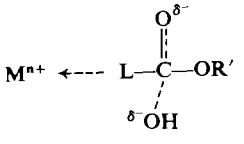
The reaction of triethanolamine (TEA) with active substrates—*p*-nitrophenyl esters and cinnamoyl imidazole (CI)—is catalyzed by divalent heavy metal ions. With Hg^{2+}, rate enhancements of 100–1000 (depending on the substrate) were observed, the overall rate constants of substrate decomposition thus exceeding those of spontaneous hydrolysis up to 100,000-fold. The predominant active species at low L:M ratio was found to be the Hg-$(TEA)_2$ complex. The dependence of the reaction rate upon excess of amino alcohol—at constant Hg^{2+} concentration—is attributable to formation of another active complex—Hg-$(TEA)_3$.

The high reactivity of the system is due to the alcoholate group of metal-bound TEA, whose p*K* has been lowered by the proximity of the metal ion. This labile nucleophilic alcoholate attacks the substrate causing its alcoholysis and forming *O*-acyl-TEA. The lability of the metal–alcoholate bond can be enhanced by low concentrations of halide ions, thus causing up to 5-fold additional increase in alcoholysis rate. Higher halide ion concentrations cause inhibition, probably due to formation of inactive HgX_2 molecules.

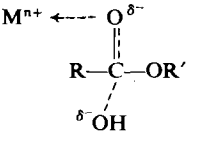
Presumably an important role of the metal ion in metalloenzymes is to affect the decrease in the p*K* value of a reactive group so that it can exhibit activity under physiological conditions.

Metal ions can catalyze hydrolytic and nucleophilic reactions by various mechanisms:

1. A positively charged metal ion introduced in a position near the susceptible bond in the substrate may act as an "electron pump," (*1*) reducing the electron density developing on the carbonyl of the substrate in the course of the attack by the hydroxide ion. This type of effect can also be produced by other positively charged groups in the substrates, such as protonated amino groups (*2*). The metal ion acts in this case as a Lewis acid, stabilizing the negatively charged transition state. This stabilization can occur via an inductive mechanism through the acyl moiety of the substrate (I) (L being a ligand group), or by polarization of the carbonyl bond (II):



I



II

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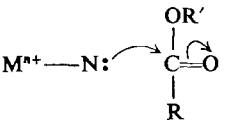
2. The metal ion (as such or in chelate form) can act as a carrier of hydroxide ions (*3*) or other nucleophilic reagents $\ddot{N}$. This would increase the concentration of the reactive species of the nucleophile N: at neutral pH (III).



III

Since in this case the reaction proceeds by direct transfer of the nucleophile from the metal ion to the substrate, it is also facilitated by the fact that the desolvation process of the bound nucleophile requires less energy than that of a free one [*4*].

3. Another possibility is that the metal ion stabilizes a labile intermediate [*1*] or product in the course of the reaction, even if it does not interact directly with the substrate itself. This might also affect the equilibrium of the reaction, by pushing it toward products. In some cases these reactions are not truly catalyzed but only "promoted" [*1*], since the metal ion is bound more strongly to the product than to any other species, and the active species is "consumed" by the reaction promoted by it, instead of being regenerated.

Similar mechanisms may also be operative by the metal ions of hydrolytic metalloenzymes, and their elucidation will, therefore, shed light on the mechanism of action of this class of enzymes.

Tertiary amino alcohols were found in a previous study to enhance the rate of decomposition of active substrates up to 100-fold [*5*], the initial product being an *O*-acyl amino alcohol. It was discovered that heavy metal ions, in particular Hg^{2+}, catalyze this reaction, leading to acylation rates 100–1000 times higher than in the absence of metal ions.

A similar finding, where an enhanced transesterification of a *p*-nitrophenyl ester to the hydroxyl group of the complex Zn^{2+}–hydroxyethyl-ethylenediamine took place, has been recently described [*6*]. However, in that study the substrates were also bound to the central metal ion. In our study the reaction kinetics of metal–TEA[3], and in particular Hg^{2+}–TEA, with *p*-nitrophenyl esters 1 and *trans*-cinnamoyl imidazole (CI) was studied and analyzed.

$XCH_2COOC_6H_4NO_2$

X = H— pNPA X = CH_3CONH— NPAG

X = CH_3COO— NPCMGO X = $C_6H_5CH_2OCONH$— NPZG

1

EXPERIMENTAL

Materials

Reagent-grade inorganic materials were used. $Hg(NO_3)_2$ was from Baker (Analyzed Reagent). Triethanolamine (TEA) was from Fluka (puriss). The substrates—pNPA,

[3] Abbreviations used: TEA, triethanolamine; CI, *trans* cinnamoyl imidazole; *p*NPA, *p*-nitrophenyl acetate; NPAG, *p*-nitrophenyl acetylglycinate; NPZG, *p*-nitrophenyl carbobenzoxy glycinate; NPCMGO, *p*-nitrophenyl carbomethoxyglycolate.

NPCMGO, and CI—were from a previous study (*5*). *p*-Nitrophenyl acetylglycinate (NPAG) was synthesized according to a general method (*5*) and had the following characteristics: mp 120° [lit. 117° (*7*)], ester content 99 %. *p*-Nitrophenyl carbobenzoxyglycinate (NPZG) was from Yeda (mp 124°, >98 %).

Deionized fresh glass-distilled water was used for the solutions.

Methods

Amino alcohols and their solutions were as described previously [*5*]. Stock solutions of 0.1 *N* $Hg(NO_3)_2$ were protected against hydrolysis by addition of 0.025 *N* HNO_3, and their Hg^{2+} content was determined by volumetric titration with EDTA, using xylenol orange as indicator (*8*). Solutions of the metal ion–ligand complexes were freshly prepared for each kinetic run. It was generally found preferable to add the components in the following order: the ligand, the required volume of NaOH to bring the solution to the desired pH value, the nitrate salt of the metal ion, the complementary amount of $NaNO_3$ needed to maintain constant ionic strength, and the buffer when necessary. The kinetic runs were carried out at 30° and the ionic strength was usually kept at 0.12.

Kinetics was measured spectrophotometrically by following the disappearance of substrate in the case of CI or appearance of product in the case of *p*-nitrophenyl esters (*5*). The amino alcohols served usually both as ligands and as buffers (*5*). The reactions were initiated by the addition of 0.05 ml of substrate solution ($2–6 \times 10^{-3}$ *M* in acetonitrile) to 2.45 ml of complex solution (*5*). Complex concentrations (1×10^{-3}–1×10^{-2} *M*) were in large excess over substrate concentrations, so that pseudo-first-order kinetics prevailed. There was no effect on the observed first-order rate constant by varying substrate concentration (3×10^{-5}–1.2×10^{-4} *M*), and in several experiments performed at much higher substrate concentration (3×10^{-3} *M*)—by the aliquots method (*5*)—the rate constants were lowered by less than 10 %. Rates were followed usually up to seven half-times and final absorbance values determined in all cases (*5*). The data were reproducible within 5 %. The nature of the product was determined by the hydroxamic acid method for esters in the case of *p*-nitrophenyl esters and spectrophotometrically in the case of CI (*5*).

Calculations

k_{obs} is the observed pseudo-first-order rate constant,

$$d[S]/dt = -k_{obs}[S] \tag{1}$$

In the absence of metal ions and ligand the spontaneous rate constant is k_0:

$$k_0 = k_{OH}[OH^-] \text{ (k_w the rate due to } H_2O \text{ was practically zero).} \tag{2}$$

In the presence of TEA

$$k_{obs} = k_0 + k'_{TEA}[TEA] \tag{3}$$

k'_{TEA} is the pH-dependent second-order rate constant for the reaction of TEA with the substrate at the pH studied (*5*).

In the presence of metal complex, corrections were applied for the contribution of other species to the rate. It was assumed that two TEA molecules are bound to each Hg^{2+}

ion (the binding constants $\log K_1 = 6.18$, $\log K_2 = 6.90$ (*9*) ensure better than 95% binding in the concentration range studied). These corrections, which are generally small (less than 5% of k_{obs}) yielded k_{cor} values:

$$k_{cor} = k_{obs} - k_0 - k'_{TEA}([TEA)_T - 2[Hg^{2+}]_T), \quad (4)$$

the subscript T stands for the total concentration of the species.

Second-order rate constants could also be obtained for the various complex species by plotting k_{cor} values versus complex concentration; for example for the Hg-$(TEA)_2$ complex

$$k_{cor} = k'_{ML_2}[ML_2]. \quad (5)$$

RESULTS

Active substrates undergo rapid alcoholysis by TEA (*5*). In the presence of divalent metal ions such as Hg^{2+}, Co^{2+}, and Cu^{2+} the alcoholysis rate constants are enhanced by factors ranging from 100 to 1000.

The dependence of the reaction rate of Hg–TEA with active substrates on the concentration of Hg^{2+} at pH 8 and at two M:L ratios—1:2 and 1:10—is shown in Fig. 1.

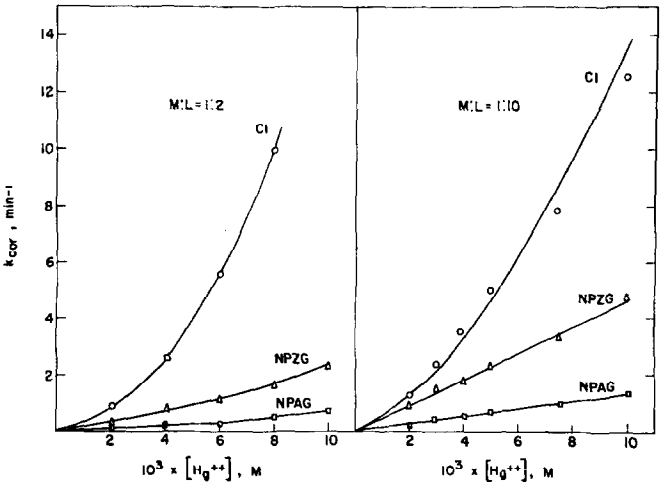


FIG. 1. Rate constants of decomposition of various substrates vs [Hg–(TEA)], pH 7.95, $I = 0.12$, $T = 30°$. M:L = 1:2 left, M:L = 1:10 right.

The slopes of these plots (or, in the cases of upward curvature, the initial slopes) were taken as the apparent k values for the complex Hg–TEA and are presented in Table 1, together with the rate constants for the Co^{2+} and Cu^{2+} complex-catalyzed reactions.

Since in the case of Hg–TEA the ML_2 complex is fully formed, the enhanced reaction rate is most probably due to this species.

$$ML_2 + S \xrightarrow{k'_{ML_2}} MLL' + P \quad (6)$$

L′ is acylated ligand (see Discussion)

TABLE 1

EFFECT OF METAL IONS ON THE RATE OF ALCOHOLYSIS OF ACTIVE SUBSTRATES BY TEA, pH 7.95, $I = 0.12$, $T = 30°$

Substrate	NPAG	NPZG	NPCMGO	CI
k_0 (min^{-1})	0.113	0.013	0.074	0.012
k'_{TEA} ($min^{-1}\ M^{-1}$)	0.33	0.43	2.08[a]	3.8[a]
k'_{Hg-TEA},[b] M:L = 1:10 ($min^{-1}\ M^{-1}$)	118	455	—	900[c]
k'_{Co-TEA}, calcd for ML[d] ($min^{-1}\ M^{-1}$)	160 ± 40	143 ± 30	660 ± 180	170 ± 30
k'_{Cu-TEA}, calcd for ML[e] ($min^{-1}\ M^{-1}$)	—	—	—	331

[a] $I = 0.50$ in this case.

[b] Slopes of linear plots of k_{cor} vs $[Hg^{2+}]_T$.

[c] Approximate values from the initial slopes of the upward-curved concentration dependence of the rates.

[d] The active species was ML, a 1:1 complex between ion and ligand. Since the stability constant of Co–TEA is very low ($\log K = 1.73$) (*10*), high TEA:Co ratios had to be used to permit formation of significant amounts of complex resulting in considerable corrections.

[e] In this 1:1 complex, $\log K = 4.3$ (*10*) 98% of metal ion was chelated under experimental conditions (M:L = 1:4).

However, since even at M:L = 1:2 the rate increases faster than complex concentration (Fig. 1) it is suggested that a second-order reaction with respect to ML_2 is also operative:

$$-d[S]/dt = k'_3[ML_2]^2[S] \tag{7}$$

A plausible mechanism is the attack of the substrate by two molecules of ML_2, possibly a dimer $(ML_2)_2$:

$$2ML_2 \overset{K}{\rightleftharpoons} (ML_2)_2 \tag{8a}$$

$$(ML_2)_2 + S \xrightarrow{k_{(ML_2)_2}} ML_2 \cdot MLL' + P \tag{8b}$$

The apparent third-order rate constant k'_3 depends, therefore, on K and $k_{(ML_2)_2}$.

An alternative mechanism, binding of the substrate to one molecule of ML_2 and attack on ML_2S by a second ML_2 molecule

$$ML_2 + S \rightleftarrows ML_2S \tag{9a}$$

$$ML_2S + ML_2 \rightarrow 2ML_2 + P \tag{9b}$$

was not consistent with the kinetic data.

The rate constants for the species ML_2 and $(ML_2)_2$, derived according to these schemes, are summarized in Table 2.

TABLE 2

RATE CONSTANTS FOR THE REACTION OF Hg–TEA WITH VARIOUS SUBSTRATES AT pH 7.95 AND M:L = 1:2—CALCULATED ACCORDING TO THE MODEL OF EQS. (6) AND (7)

	NPAG	NPZG	NPCMGO	CI
k'_{ML_2} ($min^{-1}\ M^{-1}$)	20	127	133	7
k'_3 ($min^{-1}\ M^{-2}$)	5,900	8,400	8,800	156,000

Dependence of the Rate on the TEA:Hg Ratio

The reaction rate of Hg–TEA with active substrates was measured at pH 7.95 and constant Hg^{2+} concentration ($2 \times 10^{-3}\ M$) with various ligand concentrations, and k_{cor} values were plotted against L:M (Fig. 2). Since the complex ML_2 is >95% formed

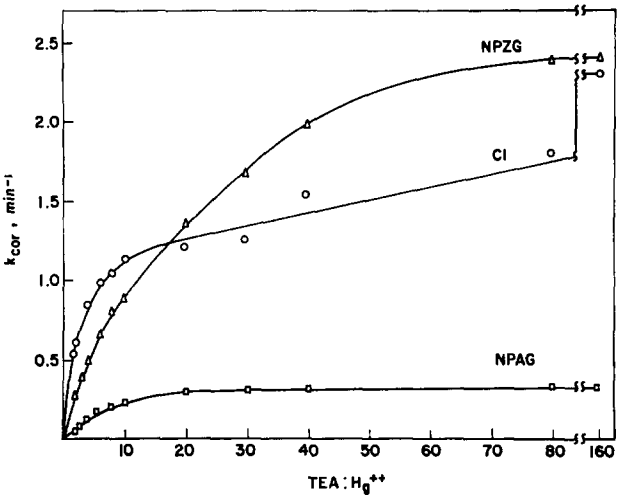


FIG. 2. Rate constants of decomposition of various substrates by Hg–TEA vs M:L ratio at pH 7.95, $T = 30°$, $I = 0.12$, $[Hg^{2+}] = 2 \times 10^{-3}\ M$.

even at M:L = 1:2, the "saturation" curves obtained indicate interaction of one of the components with excess ligand.

Presumably a new catalytically active complex ML_3 is formed in addition to ML_2, which enhances the reaction rate

$$ML_2 + L \underset{}{\overset{K_3}{\rightleftharpoons}} ML_3 \tag{10a}$$

$$ML_3 + S \xrightarrow{k_{ML_3}} ML_3 + P \tag{10b}$$

(An alternative mechanism, namely, the binding of the substrate to the ligand to form a LS complex which would react with ML_2

$$L + S \underset{}{\overset{K_{LS}}{\rightleftharpoons}} LS \tag{11a}$$

$$LS + ML_2 \rightarrow ML_2 + P + L \tag{11b}$$

is unlikely, as binding of substrate to ligand even at high concentration of the latter was never observed.)

It was assumed that the high k_{cor} value as the rate levels off is due to ML_3 species, whereas k_{cor} at M:L = 1:2 is due to ML_2. By plotting a double-reciprocal plot, i.e., reciprocal of rate increase vs reciprocal of free ligand concentration, it was possible to evaluate K_3 and k'_{ML_3} (Appendix I). Results are summarized in Table 3.

TABLE 3

RATE CONSTANTS OF THE REACTION OF Hg–TEA WITH VARIOUS SUBSTRATES, AS CALCULATED FROM THE PLOT OF k_{cor} VS M:L, pH 7.95, 30°[a]

Substrate	k'_{ML_2}[b] ($min^{-1} M^{-1}$)	k'_{ML_3}[c] ($min^{-1} M^{-1}$)	K_3[c] (M^{-1})
NPAG	30	163	130
NPZG	135	1,250	44
CI	274	670	122

[a] The rate constants were calculated on the assumption of linearity with the Hg–TEA concentration (i.e., the first-order constant was divided by the Hg^{2+} concentration).

[b] Calculated from the value of k_{cor} at M:L = 1:2.

[c] Calculated according to Eq. (4A), derived in Appendix 1.

pH Dependence

The reaction rates of Hg–TEA with several substrates were measured in the pH range 7–9 at M:L = 1:10.

The results were analyzed according to Eqs. (6), (7), and (10) and k'_{ML_2} values were found to be directly proportional to $[OH^-]$ at least between pH 7 and 9 (Table 4).

TABLE 4

pH DEPENDENCE OF THE RATE CONSTANTS FOR THE REACTION OF Hg–TEA WITH ACTIVE SUBSTRATES AT THE RATIO M:L = 1:10, $I = 0.12$, $T = 30°$

Substrate	pH	k'_{ML_2}[a]	k'_{ML_3}[b]
NPAG	7	13	
	8	118	
NPZG	7	48.5	
	8	455	
	9		4550
NPCMGO	7	50	
	9		4550

[a] According to Eq. (5).

[b] According to Eq. (10b).

Effect of Anions on the Rate of Reaction

Substitution of a halide anion Cl^- or Br^- for the normal NO_3^- anion in the medium, at constant ionic strength, affected the rate of reaction of Hg–TEA with substrates

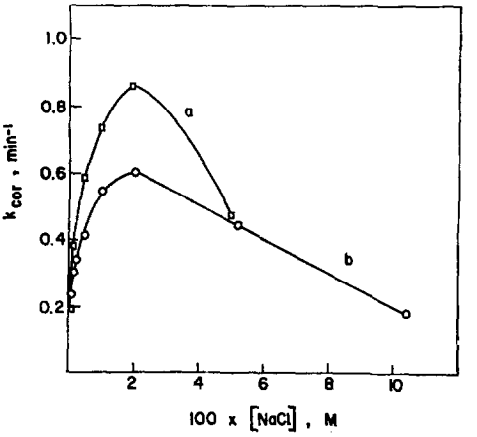


FIG. 3. k_{cor} vs concentration of chloride ions, for the reaction of Hg–TEA with NPAG at pH 7.95, $I = 0.12$, $T = 30°$. a, M:L = 1:2, $[Hg^{2+}] = 4 \times 10^{-3}$ M; b, M:L = 1:10, $[Hg^{2+}] = 2 \times 10^{-3}$ M.

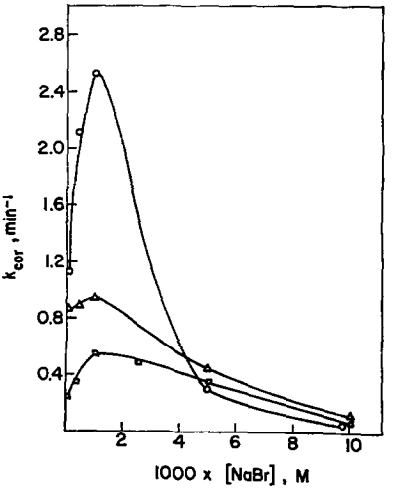


FIG. 4. k_{cor} vs concentration of bromide ion, for the reaction of Hg–TEA with NPAG ○; NPZG □; Cl △, pH 7.95, $I = 0.15$, $T = 30°$, M:L = 1:10.

(Figs. 3 and 4). Qualitatively, the same type of behavior was observed in the presence of either Cl^- or Br^-, with two important differences:

1. The concentration of halide ion causing maximum rate enhancement was significantly lower for Br^- (1×10^{-3} M) than for Cl^- (2×10^{-2} M).

2. The concentrations of Br^- causing equal inhibition of the reaction were also lower than in the case of Cl^-.

Other ions tested, fluoride and oxalate, had almost no effect on the rate of reaction. The effectiveness order of the anions $Br^- > Cl^- > F^-$ in enhancing or inhibiting the reaction is that of their binding constants with Hg^{2+} (*10*) (which is apparently not altered in the Hg–TEA complex). The following model is, therefore, suggested for the effect of halide anions on the reaction of Hg–TEA with its substrates:

$$ML^{2+} + X^- \overset{K_{X1}}{\rightleftharpoons} XML_2^+ \text{ (active)} \tag{12}$$

$$XML_2^+ + X^- \overset{K_{X2}}{\rightleftharpoons} X_2ML_2 \text{ or } X_2M + 2L \text{ (inactive).} \tag{13}$$

The kinetic behavior of the system Hg–TEA–halide anions was consistent with the suggested model. The results were analyzed according to the kinetic expression derived for the reaction of the XML_2 complex with substrates (see Appendix II) and the constants calculated are summarized in Table 5.

TABLE 5

KINETIC PARAMETERS FOR THE REACTIONS OF Hg–TEA IN THE PRESENCE OF CHLORIDE IONS CALCULATED ACCORDING TO THE MODEL OF EQS. (12) AND (13), pH 7.95, $I = 0.12$, 30°

	NPAG	NPZG	NPCMGO	CI
k'_{XML_2}, M:L = 1:2 ($min^{-1}\ M^{-1}$)[a]	209	330	1420	1845
K_{X1} (M^{-1}), M:L = 1:2	621	855	935	
K_{X2} (M^{-1}), M:L = 1:2	5.4	7.25	5.4	11.5

[a] The rate constants were calculated on the assumption of linearity with the concentration of the active species.

Product of the Reaction and its Deacylation Rate

When CI and NPAG were reacted with Hg–TEA ($10^{-2}\ M$), the reaction product was mainly ester.

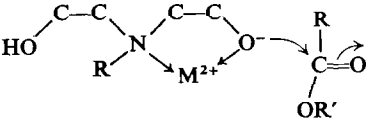
Two parallel reactions took place in solution: transesterification catalyzed by the complex, with rate constant k_{cor}, and spontaneous hydrolysis due to the direct attack of OH^- present in the solution, with rate constant k_0. Ratio of ester product to acylate ion was precisely k_{cor}/k_0.

Deacylation of the *O*-acyl-TEA proceeded at a very slow rate, which was similar to that observed in the absence of metal.

DISCUSSION

As already noted, Hg^{2+} and Cu^{2+} (Table 1) enhance the rate of acylation of TEA by a factor of 100–1000 (depending on the substrate). Since the product in this case is also an ester, some sort of interaction must take place between the metal ion and the hydroxyl groups of the amino alcohol. In Cu^{2+} complexes of TEA (and other amino

alcohols) it is generally assumed that the metal ion is chelated by the amine nitrogen and by an alkoxide anion produced by ionization of an hydroxyl group (*11*), promoted by the proximity of the metal ion. This phenomenon—increased acidity of hydroxyl groups —is also known in other metal ion complexes with amino alcohols [e.g., those of 2-hydroxyethyl-ethylenediamine with Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} (*12*)] and the pK of the hydroxyl in these complexes decreases in the following sequence: $Ni^{2+} > Co^{2+} > Zn^{2+} > Cu^{2+}$. Although the p$K$ values of hydroxyl groups in Hg^{2+} complexes of amino alcohols have not been measured, they might be even lower than their counterparts for the Cu^{2+} complexes [in analogy to the hydroxo complexes of Cu^{2+} and Hg^{2+} (*13*)]. We can thus assume for the metal ion-promoted acylation a general mechanism similar to III which does not restrict the number of hydroxyl ligands around the metal ion, nor does it include such additional features as dimerization, binding of substrate, etc., which are discussed below.

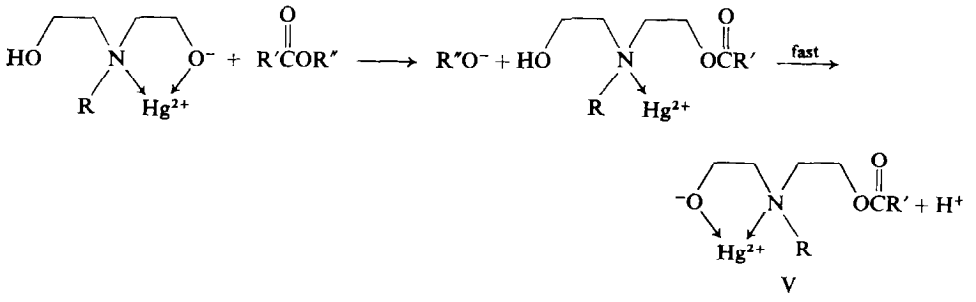


IV

The active species here contains an oxyanion which is transferred from the metal ion to the substrate carbonyl (IV). This is similar to the mechanism suggested for the reaction of hydroxo complexes of metal ions with active substrates (*3*, *13*), which leads to hydrolysis, whereas in this case alcoholysis occurs.

The ability of metal ions to exchange ligands in their coordination sphere is related to the lability of their complexes. It is known that the rate of exchange of H_2O molecules from the sphere of coordination of Hg^{2+} is very high (*14*), i.e., $Hg...OH_2$ bonds are highly labile. It should, therefore, be expected that the lability of $Hg^{2+}...OR$ bonds
H
would be even higher, since in the case of Ni^{2+}-complexes those bonds are 25 times more labile than the $Ni^{2+}...OH_2$ bonds (*15*). The high lability of Hg^{2+} complexes might also account for nonparticipation of the metal ion in the process of deacylation: the *O*-acyl-amino-alcohol is rapidly exchanged for another amino alcohol molecule, or alternatively the metal-bound esterified hydroxyl group is exchanged with a free hydroxyl group within the same molecule. Since all our experiments involved an excess of ligand (i.e., OH groups), and since free hydroxyl groups are better ligands than esterified ones, this exchange was a favored process.

The pH dependence of the reaction of the species ML_2 indicates an increase by a factor of 10 per unit of pH (from pH 7 to pH 9). This seems to agree with mechanism IV, as it might reflect ionization of one of the hydroxyl groups of TEA. However, in view of the complexity of the system, involving dimerization of the catalyst and other species of metal ligand complexes (see below), no attempt was made to determine the kinetic pK of this group. The general mechanism of the reaction may, therefore, be formulated as follows:



V

From the kinetic data (Fig. 2, Table 2) it appears that Hg^{2+} can bind a third molecule of TEA forming an active species ML_3, Eq. (10), with $\log K_3 = 2.0$.[4] ML_3 species are known to exist in TEA complexes with Ag^+ and Cd^{2+} (*16*, *17*) and are likely to be found also in the case of Hg^{2+}.

The values of k'_{ML_2} derived from the dependence on ligand concentration (Table 3) do not agree well with those calculated from the kinetic expression for the reaction at M:L = 1:2 (Table 2). This is consistent with the assumption that both ML_2 and ML_3 are active species. The finding that a pathway involving two molecules of complexes is also operative in solutions of Hg–TEA (Table 2) can be accounted for by two kinetically equivalent mechanisms:

1. Formation of an active dimeric species in a rapid preequilibrium process.
2. Attack by two Hg–TEA molecules on the substrate (with one molecule providing support for the other) in the rate-determining step.

As dimeric species with the stoichiometric composition $M_2L_2(OH)_2^{2+}$ are known to exist, at least in the case of the Cu–TEA complexes (*18*), we favor mechanism 1 also in the case of Hg–TEA. With the substrate CI, it seems that this dimeric species could account for almost the whole rate constant at M:L = 1:2, with a very small contribution by ML_2 (Table 2). The rate constant assigned to ML_2 in Table 3 may thus actually reflect the dimeric species, which seems to be the most active with respect to CI.

Although the rate constants derived for *p*-nitrophenyl esters at M:L = 1:10 (Table 1) are the slopes of straight lines, they may represent a combined attack by three active species present together in the Hg–TEA solutions, rather than a reaction involving a single species. This is suggested by the fact that these rate constants are higher than those for M:L = 1:2, but lower than would be expected if only ML_2 and ML_3 were present in the solutions, presumably due to the presence of some dimer $M_2L_2(OH)_2$, less active than ML_3. However, conditions such as the M:L ratio and Hg^{2+} concentration can be adjusted so that only one of the active species will predominate in the solution at a time.

The enhancement of reaction rates in the presence of halide anions is presumably due to formation of bonds of the type X—Hg...$^-$O (*19*) (see Eq. (12)), causing labilization of the Hg—O bond in the complex. Thus, the complexes XML_2^+ would contain a higher concentration of "dangling" alcoholate groups than the less labile ML_2 complexes. The halide ions would, therefore, facilitate transfer of alcoholate group from the central metal ion to the carbonyl group of the substrate.

[4] The variability in the values of K_3 obtained for different substrates (Table 3) might be accounted for by the fact that the correction of k_{obs} for spontaneous hydrolysis and for the ligand-catalyzed reaction amounted in some cases to 40%.

The capacity of metal ions to enhance the acylation rate of amino alcohols may usefully be applied in improving the overall catalytic efficiency in reactions involving a substrate for which deacylation is rapid while acylation with the uncomplexed ligand is rate limiting (*5*).

The features displayed by this system, and especially the lowering of pK value of reactive groups, probably represent useful pathways available to metal ions of metalloenzymes. Thus, in carbonic anhydrase, the pK of a Zn^{2+}-bound water molecule is lowered by several pH units (*20*), enabling the pH optimum for the enzyme to occur in the physiological pH range.

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APPENDIX I

Kinetic Expression for the Reaction of Amino Alcohol Complexes in the Case in Which a Third Ligand is Bound to the Metal Ion

The reaction rate in this case is given by

$$k_{cor} = k'_{ML_2}[ML_2] + k'_{ML_3}[ML_3] \tag{1A}$$

but $[ML_3] = K_3[ML_2][L]_f$ and $[ML_2] + [ML_3] = [M]_T$

since $[M]_f$ is negligible.

Also $[L]_T = 2[ML_2] + 3[ML_3] + [LH^+] + [L]_f$. Subscript T stands for total concentration and f for the concentration of free species.

Concentration of uncomplexed ligand is $[L]_0 = [L]_f + [LH^+]$ and $[L]_f = a[L]_0$, where a depends on the pK of L and on the pH of the solution (e.g., for TEA, with pK 7.80 (*5*), $a = 0.612$ at pH 8.0).

Generally $[L]_0 > [ML_3]$ hence $[L]_f \simeq a([L]_T - 2[M]_T)$. By defining $[B] = [L]_T - 2[M]_T$ and substituting $[L]_f$ for $[ML_3]$ the following equation is obtained:

$$k_{cor} = \frac{[M]_T}{1 + K_3 a[B]}(k'_{ML_2} + k'_{ML_3} a[B]) \tag{2A}$$

If all the metal ion is in the form of ML_2 the reaction rate will be

$$k'_{ML_2}[M]_T \text{ and } \Delta k \equiv k_{cor} - k'_{ML_2}[M]_T = \frac{[M]_T K_3 a[B]}{1 + K_3 a[B]}(k'_{ML_3} - k'_{ML_2}) \tag{3A}$$

$$\frac{1}{\Delta k} = \frac{1}{[M]_T K_3 a[B](k'_{ML_3} - k'_{ML_2})} + \frac{1}{[M]_T(k'_{ML_3} - k'_{ML_2})}. \tag{4A}$$

When a series of reactions is run with constant $[M]_T$ and varying ligand concentration, plotting of $1/\Delta k$ vs $1/a[B]$ yields a straight line with intercept on the abscissa $1/K_3$ and intercept on the ordinate $1/[M]_T(k'_{ML_3} - k'_{ML_2})$. An independent determination of either k'_{ML_2} or k'_{ML_3} allows the evaluation of the other constant.

APPENDIX II

Kinetic Treatment of the Reactions of Hg–TEA in the Presence of Halide Ions

Activation

The general kinetic expression for the catalyzed reaction is

$$k_{cor} = k_X + k'_{ML_2}[ML_2], \tag{5A}$$

where $k_X = k'_{XML_2}[XML_2]$

According to Eq. (12): $K_{X1} = [XML_2]/([X]_f[ML_2])$

Hence,

$$k_{cor} - k'_{ML_2}[ML_2] = k'_{XML_2}[XML_2] = k'_{XML_2}[ML_2][X]_f K_{X1} \tag{6A}$$

In solving we assumed that only the active species XML_2 is present at maximal activation by halide ions (see Fig. 3), i.e., $k'_{XML_2} = k_{cor}$, max/$[M]_T$; at other concentrations of X, there is also a contribution to the rate by ML_2. The following iterative procedure was used accordingly: (a) The contribution to rate by the active species ML_2 (calculated from step c) was subtracted from the value of k_{cor}; (b) the concentration of XML_2 was calculated: $[XML_2] = k_X/k'_{XML_2}$; (c) the "new" concentration of ML_2 was calculated: $[ML_2] = [M]_T - [XML_2]$.

The iterative process was continued until the "new" concentrations of ML_2 were identical with those used in the preceding step. We then calculated also $[X]_f = [X]_T - [XML_2]$, and obtained a value of K_{X1} for each concentration.

Inhibition

Assuming that at $[X]_f > [M]_T$, $[M]_T = [XML_2] + [X_2ML_2]$ the rate is given by $k_{cor} = k'_{XML_2}[XML_2]$.

According to Eq. (13) $K_{X2} = [X_2ML_2]/([X]_f[XML_2])$, hence, the extent of inhibition is $\Delta k = k_{cor,\,max} - k_{cor}$. After substituting, $\Delta k = k'_{XML_2}([M]_T - [XML_2])$ or

$$\Delta k/k_{cor} = k'_{XML_2}[X_2ML_2]/(k'_{XML_2}[XML_2]) = [X_2ML_2]/[XML_2] = K_{X2}[X]_f. \quad (7A)$$

Plotting $\Delta k/k_{cor}$ against $[X]_f$ yields K_{X2} as the slope of a straight line.