Metal-hydroxide-promoted Hydrolysis of 1-Acetylimidazole and its Relevance to Carbonic Anhydrase Activity

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The hydrolysis of 1-acetylimidazole in the presence of exchange-inert metal complexes, $[Co(NH_3)_5(OH)]^{2+}$, $[Cr(OH_2)_5(OH)]^{2+}$, and $[Cr(NH_3)_5(OH)]^{2+}$, has been studied in aqueous solution at 25.0 °C and I = 1.0 mol dm^{-3} ($Na[ClO_4]$). The processes appear to be predominantly nucleophilic in character as demonstrated by the reaction of $[Co(NH_3)_5(OH)]^{2+}$ with the amide where $[Co(NH_3)_5(OCOCH_3)]^{2+}$ is isolated in 93% of the theoretical yield. By analogy with the corresponding reactions of the 1-acetyl-3-methylimidazolium cation, a mechanism is featured favouring acetylimidazolium ion and metal hydroxide as the kinetically significant species. These aspects are discussed in relation to possible mechanisms for carbonic anhydrase activity.

CATALYSIS by the zinc metalloenzyme carbonic anhydrase (CA) of the reversible hydration of CO_2,[1] the hydration of aldehydes,[2] and of the hydrolysis of certain activated carboxylic [3,4] or phosphoric acid esters [5] is dependent on the ionization of a functionality in the enzyme of pK *ca.* 7. The basic form of the enzyme is the active agent in each case, and although the various catalytic reactions are closely related the identity and precise role of the catalytic group remains equivocal at the present time. There is strong evidence suggesting that it is near, or co-ordinated to, the zinc ion in the active-site cavity, and ionizations of a co-ordinated water molecule [1,6] or imidazole residue on the metal,[7] or of a nearby histidyl imidazolium group,[3,4] have been considered. Various mechanisms based on the direct or indirect (general-base) involvement of these groups have been proposed.

Despite the failure to detect an acetylimidazole intermediate in the esterase reactions (CA with 4-nitrophenyl acetate [3,4]), a nucleophilic role has been considered for both the side-chain imidazole group [4] or zinc-bound imidazolate.[8] However, this would require subsequent deacylation to be very rapid, and it would appear that the only remaining catalytic group capable of achieving this is zinc-bound water or hydroxide.

Accordingly, as a possible model for the deacylation reaction, we have examined the effects of non-labile metal aqua- (M–OH_2) and metal hydroxo- (M–OH) species on the hydrolysis of 1-acetylimidazole and the 1-acetyl-3-methylimidazolium cation. The use of exchange-inert complex ions allows a ready distinction between nucleophilic and general acid–base-catalysed processes and the role of the metal ion is clearly defined.

EXPERIMENTAL

1-Acetylimidazole was prepared by the method of Boyer,[9] and 1-acetyl-3-methylimidazolium chloride according to the procedure of Wolfenden and Jencks.[10] The aqua-complexes $[Co(NH_3)_5(OH_2)][ClO_4]_3$ [11] and $[Cr(NH_3)_5(OH_2)][NO_3]_3 \cdot [NH_4][NO_3]$ [12] were prepared using previously published methods. Treatment of a solution of the latter complex (17 g) in water (200 cm^3) with sodium perchlorate gave $[Cr(NH_3)_5(OH_2)][ClO_4]_3$ which was filtered off, washed with ethanol, and dried (Found: H, 4.00; N, 15.5. Calc. for $H_{17}Cl_3CrN_5O_{13}$: H, 3.80; N, 15.45%). Hexa-aquachromium(III) perchlorate was supplied by Research Organic/Inorganic Chem. Corp. and its solutions were standardized by atomic absorption spectrometry. All other reagents were of AnalaR grade or equivalent.

$[Co(NH_3)_5(O_2CCH_3)][ClO_4]_2$.—Freshly prepared 1-acetylimidazole (0.3 g) was added to a solution of $[Co(NH_3)_5(OH_2)][ClO_4]_3$ (1.5 g) in water (30 cm^3) adjusted to pH 6.2 (Na[OH]). After 5 min the reaction mixture was cooled in ice and the crude product filtered off. Recrystallization (water, 10 cm^3; 70 °C) gave the pure acetato-complex [0.5 g; $\lambda_{max.}$ 501 (ε 70.2), 352 nm (57.5 dm^3 mol^{-1} cm^{-1})] (Found: C, 6.00; H, 4.45; N, 17.15. Calc. for $C_2H_{18}Cl_2CoN_5O_{10}$: C, 5.95; H, 4.50; N, 17.4%).

Kinetic Measurements.—All reactions were followed spectrophotometrically using a Gilford 2400 instrument. Rate processes were normally monitored at 245 nm except for the reactions of penta-ammineaquacobalt(III) perchlorate and penta-ammineaquachromium(III) perchlorate with 1-acetylimidazole where wavelengths of 300 and 275 nm respectively were employed.

A variety of buffers (1×10^{-2}—5×10^{-2} mol dm^{-3}) was employed in the determination of the pH–rate profiles for hydrolysis of 1-acetylimidazole and 1-acetyl-3-methylimidazolium ion. Acetate, formate, hexafluoroacetone hydrate, morpholine, and *N*-methylimidazole all showed evidence of buffer catalysis. In these systems the rate at a given pH was obtained from determinations using a number of buffer concentrations, and extrapolation of the results to zero buffer concentration. Metal-ion-promoted processes were studied with the metal ion in excess using the buffering capacity provided by the $[M(OH_2)]^{3+} \rightleftharpoons [M(OH)]^{2+}$ equilibrium.

pH Measurements of kinetic solutions were carried out using a Radiometer 26 pH meter equipped with G202B and K401 electrodes. Contact of the reference electrode with the test solution was provided through a salt bridge (1.6 mol dm^{-3} $[NH_4][NO_3]$, 0.20 mol dm^{-3} $Na[NO_3]$). Electrode standardization was carried out with phthalate (pH 4.01, 25 °C) and borate (pH 9.18, 25 °C) buffers. Hydroxide-ion concentrations of solutions were calculated from measured pH values using pK_w = 14.00 and a mean molar activity coefficient of 0.67 (25.0 °C, I = 1.0 mol dm^{-3}, $Na[ClO_4]$).

Ionization Constants.—The pK_a values of $[Cr(OH_2)_6]^{3+}$ and $[Cr(NH_3)_5(OH_2)]^{3+}$ were determined, by potentiometric titration of solutions of the perchlorate salts, as 4.32 and 5.20 respectively (25.0 °C, I = 1.0 mol dm^{-3}, $Na[ClO_4]$). In cal-

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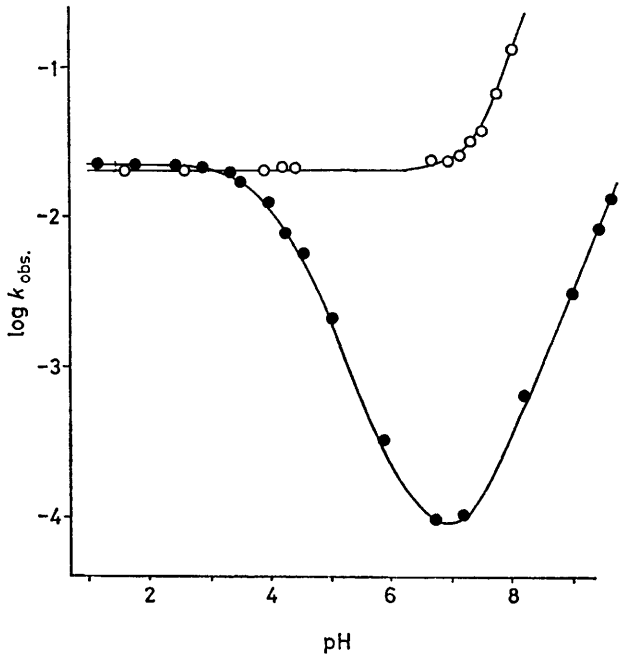
culating the extent of dissociation of the $[Co(NH_3)_5(OH_2)]^{3+}$ ion its pK_a was taken as 6.31 under the same conditions.[13]

RESULTS AND DISCUSSION

The hydrolysis rates of 1-acetylimidazole (aim) and the 1-acetyl-3-methylimidazolium ion ($amim^+$) as a function of pH at 25 °C and I = 1.0 mol dm^{-3} ($Na[ClO_4]$) are displayed in the Figure. Experimental points are given together with the profiles calculated from the rate expressions (1) for acetylimidazole hydrolysis and (2) for hydrolysis of the cation. In establishing rate law (1) we estimate pK_a ($aimH^+$) = 3.95 and values for the rate constants k_1, k_2, and k_3 are 2.22 × 10^{-2} s^{-1}, 4.6 × 10^{-5} s^{-1}, and 1.97 × 10^2 dm^3 mol^{-1} s^{-1} respectively. For hydrolysis of $[amim]^+$, k_1' = 1.97 = 10^{-2} s^{-1} and k_2' = 4.40 × 10^4 dm^3 mol^{-1} s^{-1}. Identical rate laws for the hydrolysis of the two substrates have been found in earlier studies [10,14] at 25.0 °C, however a meaningful comparison of rate constants is impracticable since a different reaction medium was employed (I = 0.2 mol dm^{-3} NaCl).

$$\text{rate} = k_1[\text{aimH}^+] + k_2[\text{aim}] + k_3[\text{aim}][OH^-] \quad (1)$$

$$\text{rate} = k_1'[\text{amim}^+] + k_2'[\text{amim}^+][OH^-] \quad (2)$$



Hydrolysis rates of 1-acetylimidazole (●) and 1-acetyl-3-methylimidazolium chloride (○) as a function of pH at 25.0 °C and I = 1.0 mol dm^{-3} ($Na[ClO_4]$). Substrate concentrations 1 × 10^{-4} (aim) and 2 × 10^{-4} mol dm^{-3} ($amim^+$). Buffers used were (pH ranges given parenthetically): $HClO_4$ (1.7—2.98), formate (3.36—4.20), acetate (4.55—5.04), hexafluoroacetone hydrate (5.87—7.17), and morpholine (8.22—9.59) for aim hydrolysis. Similar buffers were employed for hydrolysis of $[amim]^+$ except that 1-methylimidazole–$HClO_4$ solutions were used in the range pH 6.07—8.03

The addition of 1-acetylimidazole to a solution of $[Co(NH_3)_5(OH_2)][ClO_4]_3$ adjusted to pH 6.2 results in the rapid formation of the corresponding acetato-product which was isolated as its perchlorate salt. The kinetics of this process was studied in the range pH 5.80—7.03 (25 °C, I = 1.0 mol dm^{-3}, $Na[ClO_4]$) and reactions were monitored over at least 3 half-lives. Relevant rate data are included in the Table. The metal-ion-promoted contribution to the observed rate constants is given by equation (3) with k_{MOH} = 14.9 dm^3 mol^{-1} s^{-1}. $[M]_T$ and

$$k_{obs.}' = \frac{k_{MOH}K_1[M]_T[aim]_T a_{H^+}}{(K_1 + a_{H^+})(K_2 + a_{H^+})} \quad (3)$$

$[aim]_T$ represent the total concentrations of metal ion and 1-acetylimidazole respectively, and K_1 and K_2 refer to the separately determined acid-ionization constants of the aqua-ion (K_1 = 4.90 × 10^{-7} mol dm^{-3}) and acetylimidazolium ion (K_2 = 1.12 × 10^{-4} mol dm^{-3}).

Similarly, hydrolyses carried out using $[Cr(OH_2)_6]^{3+}$ (K_1 = 4.79 × 10^{-5} mol dm^{-3}) and $[Cr(NH_3)_5(OH_2)]^{3+}$ (K_1 = 6.31 × 10^{-6} mol dm^{-3}) in place of the pentaammineaquacobalt(III) ion also follow equation (3) with k_{MOH} = 4.2 and 1.6 dm^3 mol^{-1} s^{-1} respectively. The kinetically equivalent forms of the rate law for these processes [equations (4) and (5)] suggest that the results can be interpreted in terms of a reaction of protonated acetylimidazole with metal hydroxide or, alternatively, as a reaction of the free-base form of the amide with metal aqua-ion.

$$\text{rate} = k_{MOH}[\text{aimH}^+][M(OH)^{2+}] \quad (4)$$

$$= k_{MOH}K_1[\text{aim}][M(OH_2)^{3+}]/K_2 \quad (5)$$

Evidence has been previously presented [10] indicating that 1-acetyl-3-methylimidazolium ion is a satisfactory model for the reactions of the conjugate acid of 1-acetylimidazole. In the present case the reaction of the former substrate with hexa-aquachromium(III) ion in the range pH 3.89—4.43 (25 °, I = 1.0 mol dm^{-3}, $Na[ClO_4]$), Table, follows the rate law (6) with k_{CrOH} = 3.5 dm^3 mol^{-1} s^{-1}.

$$\text{rate} = k_{CrOH}[\text{amim}^+][Cr(OH)^{2+}] + k_1'[\text{amim}^+] \quad (6)$$

A comparison of this value with that calculated on the assumption that the kinetically significant metal species in the corresponding reaction of 1-acetylimidazole is the $[Cr(OH_2)_5(OH)]^{2+}$ ion (4.2 dm^3 mol^{-1} s^{-1}) strongly supports the view that metal aqua-ions are ineffective in all these processes.

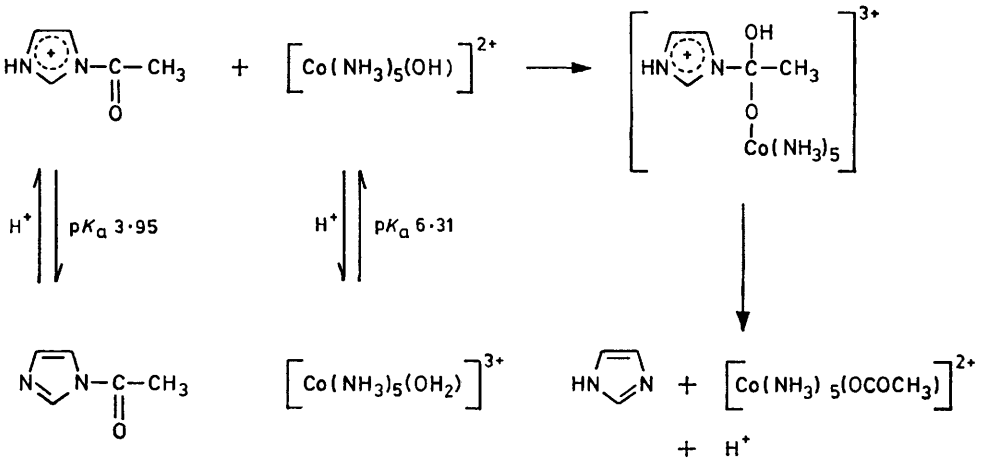
To establish the pathway for hydrolysis of 1-acetylimidazole in the presence of $[Co(NH_3)_5(OH)]^{2+}$, freshly prepared amide (0.0696 g, 6.32 × 10^{-4} mol) was added to a solution of $[Co(NH_3)_5(OH_2)][ClO_4]_3$ (2.80 g) in water (55 cm^3) adjusted to pH 6.00 ($Na[OH]$) and I = 1.0 mol dm^{-3} ($Na[ClO_4]$), and the temperature maintained at 25 °C for 75 min. The reaction was then quenched (pH 2, $HClO_4$) and the solution sorbed on Sephadex CM-C25 ion-exchange resin. On elution with sodium chloride solution (0.25 mol dm^{-3}) the recovery of $[Co(NH_3)_5(OCOCH_3)]^{2+}$ (5.62 × 10^{-4} mol), as estimated spectrophotometrically at 501 nm, amounted to 93% of that expected after due allowance had been made for hydrolysis through paths not involving the metal ion. No acetato-product was detected when a solution 0.1 mol

Kinetics of the metal-hydroxide-promoted hydrolyses of *N*-acetylimidazole and 1-acetyl-3-methylimidazolium chloride at 25 °C and I = 1.0 mol dm^{-3} (Na[ClO$_4$])

pH	10^2[M]$_T$ / mol dm^{-3}	10^2[M(OH)] / mol dm^{-3}	$10^3k_{obs.}$ / s^{-1}	10^3k_{hyd} [b] / s^{-1}	$10^3k_{calc.}$ [c] / s^{-1}
(*i*) Acetylimidazole [a]–[Cr(NH$_3$)$_5$(OH)]$^{2+}$					
4.53	2.45	0.432	5.96	4.66	6.11
4.76	2.45	6.653	4.43	3.01	4.43
5.04	2.45	1.002	2.93	1.71	2.93
5.16	2.45	1.170	2.43	1.33	2.43
5.44	2.45	1.557	1.65	0.764	1.58
(*ii*) Acetylimidazole [d]–[Cr(OH$_2$)$_5$(OH)]$^{2+}$					
3.62	2.0	0.332	23.4	15.1	24.6
3.82	2.0	0.480	23.6	12.7	24.3
3.98	4.0	1.252	34.5	10.7	36.1
4.16	4.0	1.632	33.5	8.5	34.7
4.31	2.0	0.990	20.6	6.7	19.3
4.44	2.0	1.138	19.0	5.4	17.1
(*iii*) Acetylimidazole [a]–[Co(NH$_3$)$_5$(OH)]$^{2+}$					
5.80	4.0	0.945	2.32	0.354	2.43
5.80	8.0	1.89	4.41	0.354	4.35
6.00	3.5	1.15	1.78	0.242	1.77
6.00	7.0	2.30	3.39	0.242	3.30
6.24	2.5	1.15	1.15	0.165	1.05
6.24	5.0	2.30	2.11	0.165	1.93
6.56	2.5	1.60	0.674	0.111	0.696
6.56	5.0	3.20	1.36	0.111	1.28
7.03	2.5	2.10	0.292	0.082	0.343
7.03	5.0	4.20	0.546	0.082	0.601
(*iv*) 1-Acetyl-3-methylimidazolium ion [e]–[Cr(OH$_2$)$_5$(OH)]$^{2+}$					
3.89	2.50	0.676	42.5	19.7	43.3
3.89	1.25	0.339	31.1	19.7	31.6
4.20	2.50	1.077	57.7	19.7	57.4
4.20	1.25	0.538	39.6	19.7	38.5
4.43	2.50	1.407	67.4	19.7	68.9
4.43	1.25	0.704	44.3	19.7	44.3

[a] Substrate concentration 5 × 10^{-4} mol dm^{-3}. [b] k_{hyd} represents the contribution to the rate through pathways not involving the metal ion and was calculated from equations (1) with (2) as appropriate using the values of the constants given in the text. [c] Calculated using equations (1) with (3) or (2) with (6) as appropriate and the values of the constants given in the text. [d] Substrate concentration 1 × 10^{-4} mol dm^{-3}. [e] Substrate concentration 2 × 10^{-4} mol dm^{-3}.

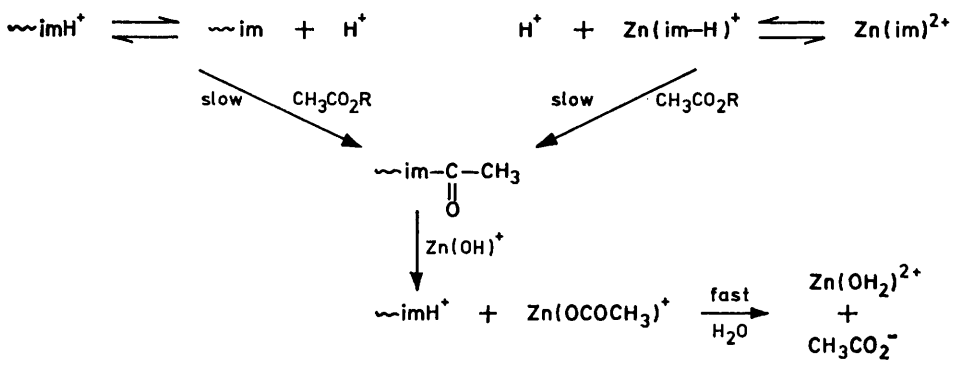
dm^{-3} in [Co(NH$_3$)$_5$(OH)]$^{2+}$ and 0.005 mol dm^{-3} in sodium acetate was allowed to stand for a similar period. These data require that the reaction proceeds by a direct nucleophilic path (Scheme 1) with little or no contribution from general-base catalysis.



SCHEME 1

A nucleophilic role for metal-bound hydroxide has also been observed in the hydrolyses of propionic anhydride [13] 4-nitrophenyl acetate,[8,15] 2,4-dinitrophenyl acetate,[15] and in the hydration of CO_2.[16] Brønsted coefficients of 0.2—0.4 for these processes [17] demonstrate the lack of sensitivity of reaction rates to the basicities of the nucleophilic reagents.* A similar Brønsted slope (0.4)

* The values quoted are based on plots including the [OH]$^-$ reaction but excluding H_2O.

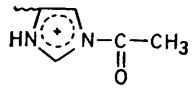


SCHEME 2

can be constructed for the $[M(OH)]^{2+}$-promoted hydrolysis of 1-acetylimidazole.* It appears that substrates possessing good leaving groups generally follow ' flatter ' Brønsted slopes rather than the more general class of oxygen or nitrogen nucleophiles ($\beta \sim 1.0$).[18]

A possible mechanism for the carbonic anhydrase-catalysed hydrolysis of active esters involving metal-ion-assisted deacetylation of an acetylimidazole intermediate is given in Scheme 2 in which the intermediate may arise from nucleophilic attack by either histidylimidazole or zinc imidazolate.

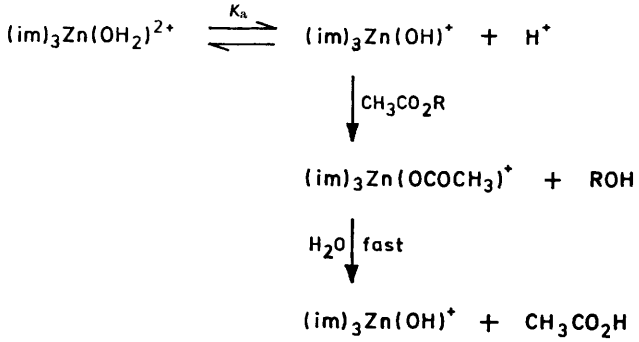
Support for such processes is lent by the observation that imidazole species (either free or co-ordinated) are more reactive (*ca.* 10^2) than $[M(OH)]^{n+}$ nucleophiles of similar basicity,[17] and by the inhibition of enzyme activity through alkylation of histidyl residues.[1] However, a major difficulty arises in the $[Zn(OH)]^+$-catalysed hydrolysis of the acylated enzyme. The present results imply that deacetylation would occur *via* the species (I) and it seems unlikely that the active site would have the capacity to provide a sufficiently high concentration of this intermediate (the expected pK_a is *ca.* 4), as well as of $[Zn(OH)]^+$, such that hydrolysis at biological pH values



(I)

is fast compared with the initial aminolysis of the ester. The active site may well act to perturb functionality acidities from their bulk-solvent values, but for maximal efficiency this would require pK_a perturbations of *ca.* +3 units for the acetylimidazolium moiety and −2 to −3 units for the metal aqua-ion (the pK_a of co-ordinated water lies in the range 9—10 for many simple zinc aqua-ions [19]). Such a situation is hard to imagine since both functionalities would need to be adjacent in the active site.

On the other hand the ' direct ' $[Zn(OH)]^+$ mechanism, Scheme 3, avoids this difficulty and it may not be a coincidence that the relative rate difference of *ca.* 10^5 found for the CO_2- and 4-nitrophenyl acetate-promoted hydrolyses by an $[M(OH)]^{n+}$ species $\{pK_a[M(OH_2)]^{(n+1)+} = 7.5$, $k_{MOH} = 4 \times 10^2$ and 3.6×10^{-3} dm^3 mol^{-1} s^{-1} respectively [17]$\}$ is also found in the enzyme ($k_{cat} = 7.1 \times 10^5$ s^{-1} for CO_2,[20] $k = 2.5$ s^{-1} for the ester [3]).



SCHEME 3

[8/1988 *Received*, 15*th November*, 1978]

* The reaction of $[amim]^+$ with $[OH]^-$ was used to establish k_{OH} for $[aimH]^+$ since the ' uncatalyzed ' reaction of aim is believed to contain little of the $[aimH]^+ + [OH]^-$ component (ref. 10).

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