Aquo-Pentamine Co(III) Complexes as Models for Carbonic Anhydrase

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ABSTRACT

The hydrolysis of 4-nitrophenyl acetate by metal complexes $Co(en)_2(imH)H_2O^{3+}$, $Co(en)_2(bzmH)H_2O^{3+}$, and $Co(en)_2(imCH_3)H_2O^{3+}$ (imH = imidazole, bzmH = benzimodazole, $imCH_3$ = methyl imidazole) has been investigated in the pH range 5.4–8.9. The small difference in nucleophilic reactivity in the pH range 5.4–6.7 is assumed to be due to hydrogen bonding abilities of the imidazole and substituted imidazole ligands and small pK_a differences (k_2(imH) = 2.2×10^{-2} M^{-1} sec^{-1}, k_2(bzmH) = 5.68×10^{-2} M^{-1} sec^{-1}, $k_2(imCH_3) = 1.35 \times 10^{-2}$ M^{-1} sec^{-1}, 40°C, 1 = 0.3 $NaClO_4$, pK_a(imH) = 6.2, $pK_a(imCH_3)$ = 6.2 and pK_a(bzmH) = 5.9). In the pH range 7.8–8.9, the differences in nucleophillic reactivity (k_3(imH) = 85.5×10^{-2} M^{-1} sec^{-1}, k_3(bzmH) = 33.4×10^{-2} M^{-1} sec^{-1}, 40°C, I = 0.3 $NaClO_4$) are reconciled with a significant steric factor outweighing the acidity of the benzimidazole complex. In the pH region 6.7–7.7, the deviation from linearity is presumably due to both hydroxo and imido ligands functioning as nucleophiles, the latter being about 40 times stronger than the former.

INTRODUCTION

Bovine carbonic anhydrase catalyzed hydrolysis of 4-nitrophenyl acetate shows evidence for two different ionizable catalytic groups differing in pK by four units [1]. These two catalytic groups have been identified to be a histidine imidazole and a water molecule, both coordinated to Zn^{2+} [1, 2]. The esterase property has been interpreted in terms of $ZnOH^{2+}$ activity at lower pH and $ZnIM^{2+}$ activity at higher pH. Recent studies of 4-nitrophenyl acetate hydrolysis by Sargeson et al. [3] with $(NH_3)_5CoIm^{2+}$ and $(NH_3)CoOH^{2+}$ as model systems have shown the former to be superior as a nucleophile in keeping with its higher basicity than the latter. However, no attempt has been made to see exactly what role is played by the coordinated imidazole at the enzyme active site at the biological pH where it is expected to exist almost in the protonated form pK_a = 10.02 [4]. This work

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attempts such an investigation with a few aquo pentamines as models, which are hoped to mimic the enzyme more adequately than the pentamines.

EXPERIMENTAL

Spectrophotometric measurements were made on EC G-866B uv and visible spectrophotometers and pH measurements were made with Phillips pH meter model 9040 equipped with a combined glass and saturated calomel electrode, standardized with standard phosphate buffer of pH 6.84 at 40°C.

4-Nitrophenyl Acetate

4-Nitrophenylacetate was prepared following the method of Chattway [5] and purified by repeated crystallization from alcohol. Stock solution was prepared by dissolving a calculated amount in acetone.

$Co(en)_2(imH)H_2O^{3+}$, $Co(en)_2(bzmH)H_2O^{3+}$, and $Co(en)_2(imCH_3)H_2O^{3+}$

$Co(en)_3H_2O^{3+}$ were prepared by standard procedures as follows: The chloroperchlorate analogs were obtained by the method of Bailar and Clapp [6] and crystallized thrice from aqueous perchloric acid solutions, washed with ethanol and ether. The perchlorate salts were hydrolyzed with known volumes of standard alkali solutions and then acidified with perchloric acid solution to pH 4. The resulting solutions were concentrated at 60–70°C to the crystallization point and then cooled. The crystals were filtered off, washed successively with ice-cold water, ethanol, and ether, and finally stored over fused calcium chloride. They were analyzed by estimation of cobalt (found: Co 10.35, calculated for $Co(en)_2(imH)H_2O(ClO_4)_3$: 10.46; found Co 9.4, calculated for $Co(en)_2(bzmH)H_2O(ClO_4)_3$: 9.6; found Co 10.1, calculated for $Co(en)_2(imCH_3)H_2O(ClO_4)_3$: 10.2). Sodium perchlorate (Riedel) was used for ionic strength adjustments. Imidazole, benzimidazole, and *n*-methyl imidazole (Fluka A. G.) were used without further purifications. All other chemicals used were of Analar grade.

Spectral Measurements

The aquo-imidazole complex exhibited maxima at 480 nm while aquo-benzimidazole and aquo-methyl imidazole exhibited maxima at 485 nm in 0.1 *M* $HClO_4$ acid medium with molar extinction coefficients of 80.0, 84.0, and 73.0 $dm^3\ mol^{-1}\ Cm^{-1}$. These values agree satisfactorily with reported values of 81.6 ± 1.0, 92.0, and 75.5. ± 1.0 (7). The reaction products after acidification to pH 1 also exhibited maxima at the above values without any appreciable change in extinction coefficients. This confirms their catalytic role in the hydrolytic process. The pH of the reaction products were measured at 40°C at the end of at least three half-lives.

Kinetic Measurements

The kinetics of hydrolysis of 4-nitrophenyl acetate were followed at different pH by the spectrophotometric method. Reaction mixtures were prepared by neutralizing a

fixed volume of the stock complex (2.5 dm^3) solution with varying amounts of standard sodium hydroxide solution and then adjusting the ionic strength with sodium perchlorate solution. All solutions were thermostated before mixing. The resulting mixture was also thermostated. A 2×10^{-3} *M* solution of 4-nitrophenyl acetate in water was prepared from the stock solution (in acetone) and thermostated just before use. 5 dm^3 of this solution was then transferred to the complex solution which was thermostated as quickly as possible. Aliquots of 2 dm^3 of the reaction mixture were withdrawn at regular time intervals and quenched with 2 dm^3 of 0.1 *M* $HClO_4$ acid solution. 1 dm^3 of tris buffer (M/2) was added just before optical density measurements. A ratio of 10:1 was maintained between the total complex and 4-nitrophenyl acetate in all the runs. The pseudo-first-order rate constants were computed from the gradients of plots of log $(D_\infty - D_t)$ against time, where the terms have their usual meaning. For the measurements at the higher pH region, tris buffers of desired compositions were prepared by neutralizing this with standard perchloric acid solution.

RESULTS AND DISCUSSION

The pseudo-first-order rate constants for 4-nitrophenyl acetate are presented in Tables 1 and 2. In the pH range 5–7.0 the rate data are consistent with the rate equation

$$k_{obs} = \frac{k_1 + k_2 K\,[H^+]^{-1}}{1 + K\,[H^+]^{-1}} \times [\text{complex}]_T$$

which on rearrangement gives

$$\frac{[\text{complex}]_T}{k_{obs}} = \frac{1}{(k_2 - k_1)} \times \frac{[H^+]}{K} + \frac{1}{(k_2 - k_1)} \cdots \qquad (1)$$

if $k_1\,[\text{complex}]_T \ll k_{obs}$, where k_1 and k_2 are the rate constants of the steps catalyzed by aquo and hydroxo forms of the complex and K is the equilibrium constant for the equilibrium

$$Co(en)_2(L)H_2O^{3+} \overset{K}{\rightleftharpoons} Co(en)_2(L)OH^{2+} + H^+$$

where L = imH, bzmH, and $imCH_3$. In keeping with equation (1) plots of k_{obs}^{-1} vs. $[H^+]$ were found to be fairly linear for aquo-*n*-methyl imidazole complex, but considerable deviations were observed for aquo-imidazole and aquo benzimidazole complexes beyond pH 6.70 ($[H^+] = 0.20 \times 10^{-6}$) (Fig. 1). Such deviations are presumably due to ionization of $-NH$ protons to form the corresponding imido bases which function as stronger nucleophile [3]. However, extrapolations of the plots give positive intercepts in all cases. The pK values obtained from such intercepts and the slopes of the above plots are in excellent agreement with those determined experimentally from pH titrations of the above (pK values calculated 6.4, 6.2, and 6.5 for L = imH, bzmH, and $imCH_3$, respectively; experimental values – 6.2 for L = imH and $imCH_3$ and 5.9 for L = bzmH).

The pK values for the complexes suggest that the complexes would exist

TABLE 1. Pseudo-first-order Rate Constants for Hydrolysis of 4-nitrophenyl Acetate in $Co(en)_2(L)H_2O/Co(en)_2(L)OH^{2+}$ Buffers at 40°C and I = 0.3[a]

[Complex]/[NaOH]	$Co(en)_2(bzmH)H_2O^{3+}$		$Co(en)_2(bzmH)H_2O^{3+}$		$Co(en)_2(imCH_3)H_2O^{3+}$		$Co(NH_3)H_2O^{3+}$	
	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$
0.002/Nil	5.04	0.087	—	—	—	—	—	—
0.002/0.00018	—	—	5.30	0.20	5.30	0.09	—	—
0.002/0.00033	5.56	0.091	5.60	0.24	—	—	—	—
0.002/0.00056	5.79	0.113	—	—	—	—	—	—
0.002/0.001	6.18	0.16	6.02	0.357	6.00	0.10	6.00	0.014
0.002/0.00125	6.44	0.227	6.30	0.56	6.47	0.125	6.40	0.12
0.002/0.0016	6.80	0.37	6.71	1.01	6.82	0.20	6.87	0.19
0.002/0.0018	7.17	0.66	7.10	2.98	7.20	0.21	7.20	0.215
0.002/0.002	7.40	1.04	7.30	3.9	—	—	—	—
0.002/0.001 $HClO_4$	—	0.061	—	0.065	—	0.032	—	—

[a] In 0.001 $HClO_4$ alone, the rate content was found to be 0.036×10^{-4}.
All readings are mean of duplicate runs.

TABLE 2. Pseudo-first-order Rate Constants for Hydrolysis of 4-nitrophenyl Acetate in Tris/$HClO_4$ Acid Buffers at 40°C and $I = 0.3$[a]

[Tns]T/[$HClO_4$]	No. Complex		$Co(en)_2(imH)H_2O^{3+}$		$Co(en)_2(bzmH)H_2O^{3+}$		$Co(en)_2(im\text{-}CH_3)H_2O^{3+}$		$Co(NH_3)_5H_2O^{3+}$	
	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$	pH	$10^4k/sec^{-1}$
0.1/0.07	7.56	0.806	7.51	2.10	7.50	5.2	7.46	1.35	7.52	1.30
0.01/0.06	7.85	1.15	7.07	2.81	7.88	8.3	7.82	1.70	–	–
0.1/0.05	8.05	1.50	8.05	3.48	8.06	11.9	8.07	2.58	8.09	2.51
0.1/0.04	8.35	2.10	8.37	6.10	8.31	16.1	8.30	3.5	–	–
0.01/0.03	8.53	2.76	8.54	8.40	8.56	28.7	8.60	5.98	8.62	6.00
0.01/0.02	8.73	3.95	–	–	–	–	–	–	–	–
0.1/0.015	8.93	6.12	8.87	16.4	8.85	44.6	–	–	–	–

[a] All readings are mean of duplicate runs.

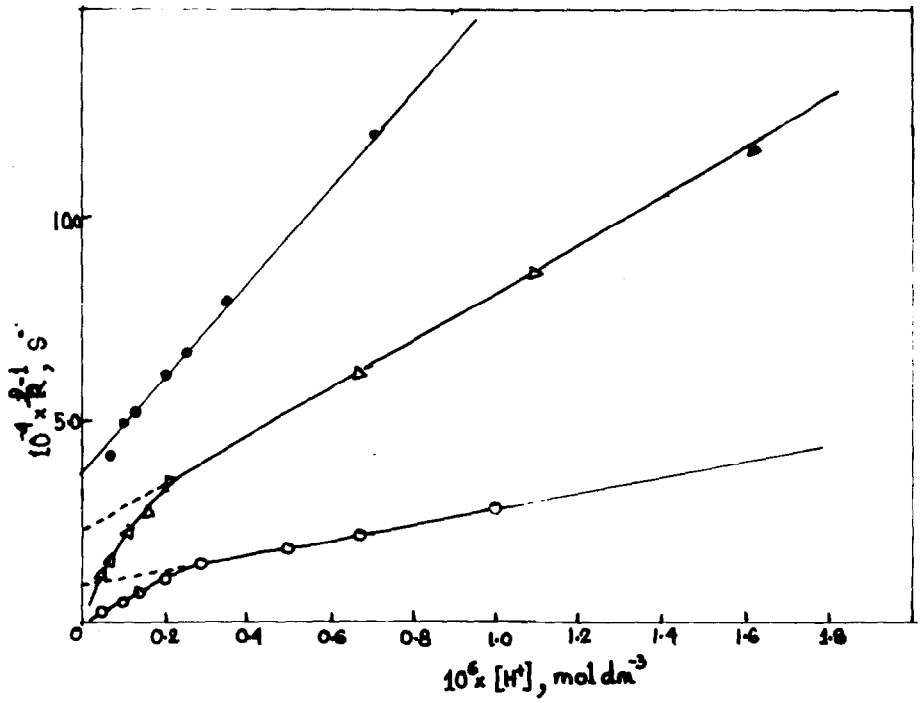

FIGURE 1. pH rate profiles for 4 NPA hydrolysis beyond pH 6.7 in the presence of [-○-○-] $Co(en)_2(im\text{-}CH_3H_2O^{3+}$; [-△-△-] $Co(en)_2(imH)H_2O^{3+}$; [-●-●-] $Co(en)_2(bzmH)\ H_2O^{3+}$.

completely in the aquo form at pH 4.5 and therefore it would be reasonable to assume that the value of k_{obs} at pH 4.5 (i.e., when no alkali is added) is k_1. This has been confirmed further by determination of rate constants in 0.00 1*M* $HClO_4$. Using these values of k_1, k_2 values were computed from the slopes of the above plots as 2.2×10^{-2} and 1.35×10^{-2} sec^{-1} for imH, bzmH, and $imCH_3$, respectively. These values can be reconciled with the hydrogen bonding abilities of the NH proton which depends upon their acid strength (pK_{NH} for bzmH $- 8.6 \pm 0.1$ [8] and imH $= 9.2 \pm 0.04$ [7]. Such hydrogen bonding by imidazole (57) has been proposed to promote the nucleophilic attack of a serine hydroxyl group (195) at the active site of the enzyme chymotripsin, on the carboxyl carbon atom of the acyl group of a substrate [9]. In the pH range 7.7–9.0, the rate data are consistent with the rate equation

$$k_{app} = (k_0 - k_0') = \frac{k_2 + k_3 K_{NH}\ [H^+\}^{-1}}{1 + K_{NH}\ [H^+]^{-1}} \times [\text{complex}]_T$$

where k_0 and k_0' are overall observed rate constants in tris buffers in the presence and absence respectively, of the complex, k_2 and k_3 are rate constants of the paths catalyzed by hydroxo imidazole and hydroxo-imidazolato (-1) forms of the complexes, and K_{NH} is the equilibrium constant for the ionization step

$$Co(en)_2(L)(OH)^{2+} \overset{K_{NH}}{\rightleftharpoons} Co(en)_2(L{-}H)(OH)^{1+} + H^+.$$

Plots of k_{app}^{-1} vs. [H^+] were linear over the pH range, k_2 [complex] being $\ll k_{app}$ (Table 3, Fig. 2). When these plots were extrapolated to lower pH (7.4), the k_{app} values were close to the values obtained in self-buffer of these complexes, as expected.

The increase in k_{app} values with increasing pH, in the case of aquo *n*-methyl imidazole complex, is possibly due to the fact that the buffer catalysis is not independent of the high total buffer concentration employed (0.1 *M*). Similar behavior was also observed with $Co(NH_3)H_2O^{3+}$. Significant buffer catalysis was observed by Fife et al. [10]. in the case of metal ion effects on intramolecular general base and nucleophilic carboxyl group. participation in ester hydrolysis.

From the slopes of the plots of k_{app}^{-1} vs. [H^+] the values of k_3 were found to be 85.5×10^{-2} M^{-1} sec^{-1} and 33.46×10^{-2} M^{-1} sec^{-1} for imidazole and benzimidazole complexes, respectively. Assuming buffer catalysis to be the same for both the complexes, it can be concluded that imidazolato (-1) is roughly 2.5 times stronger as a nucleophile than benzimidazolato (-1). This is in contrast to the smaller pk_2 value of the latter species, probably due to a considerable steric factor. (The pk_{NH} values calculated, from the slopes and intercepts of the above plots, were 8.87 for the imH complex and 8.78 for the bzimH complex.)

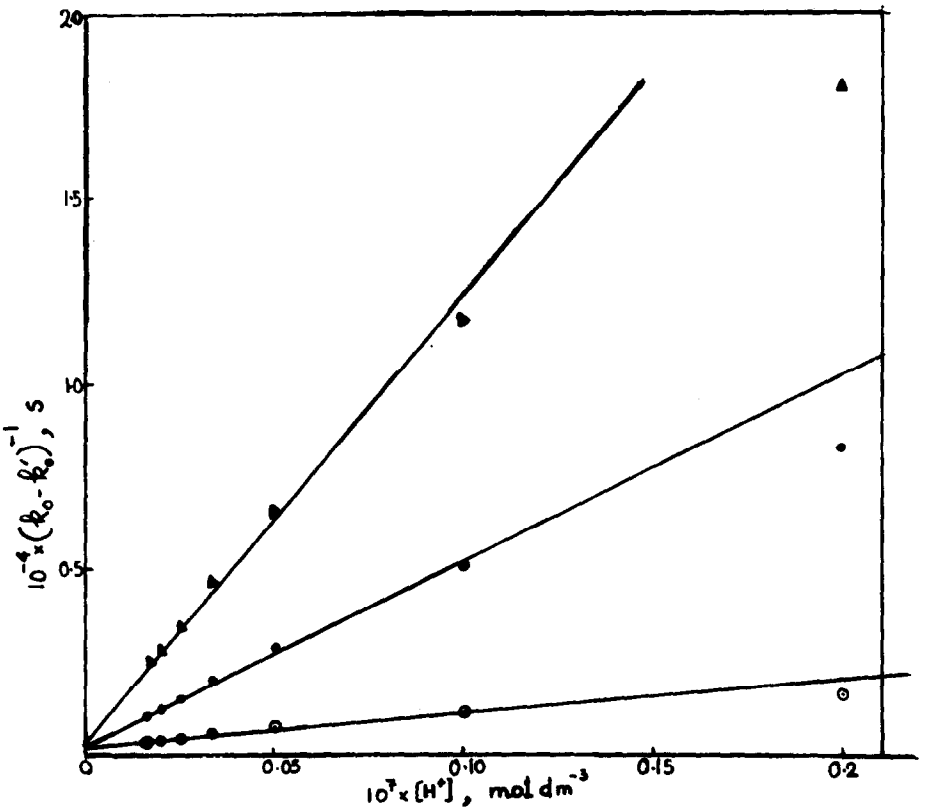

FIGURE 2. Plots of K_{app}^{-1} Vrs [H^+; [-O-O-] $Co(en)_2(bzmH)H_2O^{3+}$; [-●-●-] $Co(en)_2(imH)H_2O^{3+}$; [-Δ-Δ-] $Co(en)_2(im\text{-}CH_3)H_2O^{3+}$.

TABLE 3. Comparison of $(k_0 - k_O')$ Values for the Complex at 40°C, $I = 0.3$ Obtained from the Plot of k_{obs} vs. $(H^+)^{-1}$ and k_{obs}' vs. $(H^+)^{-1}$

Buffer Medium	$[H^+]^{-1}$ mol dm^{-3}	pH	$Co(en)_2(ImH)H_2O^{3+}$	$Co(en)_2(BzmH)H_2O^{3+}$	$Co(en)_2(imCH_3)H_2O^{3+}$
Tri5	5.0×10^{7}	7.70	1.20×10^{-4}	5.65×10^{-4}	0.55×10^{-4}
	10.0×10^{7}	8.00	1.95×10^{-4}	8.25×10^{-4}	0.85×10^{-4}
	20.0×10^{7}	8.30	3.4×10^{-4}	13.2×10^{-4}	1.50×10^{-4}
	30.0×10^{7}	8.477	4.9×10^{-4}	18.4×10^{-4}	2.10×10^{-4}
	50.0×10^{6}	8.70	7.9×10^{-4}	28.5×10^{-4}	3.50×10^{-4}

It is interesting to note that in the pH region 6.7–7.7, the kinetics of hydrolysis does not follow the first-order kinetics and both coordinated hydroxide and imidazole (−1) are active nucleophiles, though the latter is about 40 times more active than the former as has been suggested earlier [3].

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