Amine Ligand Effects in Hydroxoaqua(tetraamine)cobalt(III) Ion Promoted Hydrolysis of Adenosine 5′-Triphosphate

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Since we first reported[2] on the extraordinary reactivity of Co(trpn)(OH)(H_2O)$^{2+}$ (the most reactive form of Co^{III}(trpn)(aq))[3] in promoting the hydrolysis of phosphate esters and polyphosphates, high interest has continued in the special qualities of the trpn moiety.[4-8] Here we compare the effectiveness of Co^{III}(trpn)(aq) with three other tetraamine complexes of type Co^{III}(N_4)(aq) (N_4 = cyclen, (tn)$_2$, and tren)[3] in promoting the hydrolysis (dephosphorylation[9]) of ATP to ADP and P_i (orthophosphate) in preformed complexes of type β,γ-Co(N'_4)ATP$^-$. The order of effectiveness for these Co^{III}(N_4)(aq) complexes at pH 6.5 (trpn > cyclen > (tn)$_2$ > tren) parallels water substitution rates in the complexes, and related substitution rates such as for aquation of the corresponding Co(N_4)Cl_2^+ ions. Comparison of substitution and dephosphorylation rates provides further insight into the mechanism of the latter process.

Experimental Section

Aqueous solutions containing Co^{III}(N_4)(aq) were prepared from the corresponding carbonato[10,11] or hydroxoaqua[12,13] complexes; preparations for the following complexes have been described: [Co(trpn)CO_3]ClO_4,[4d] [Co(cyclen)CO_3]Cl,[14] [Co(tren)CO_3]ClO_4,[10,15] [Co(tn$_2$)(OH)(H_2O)](ClO_4)$_2$.[12,13,16] Sources for other chemical procedures have been given previously.[2b,11,13]

Table I. Comparison of Initial Rates of ATP Hydrolysis, v_0 (M s^{-1}), for Co(N'_4)ATP$^-$ (10^{-3} M) in the Presence of Co^{III}(N_4)(aq) (10^{-3} M) at pH = 6.5, I = 0.1 M ($NaClO_4$), and T = 25 °C

Co(N'_4)ATP$^-$	Co^{III}(N_4)(aq)	$10^9 v_0$
Co(tn$_2$)ATP$^-$	none	1.0
	Co^{III}(trpn)(aq)	6900
	Co^{III}(cyclen)(aq)	620
	Co^{III}(tn$_2$)(aq)	93.
	Co^{III}(tren)(aq)	4.1
Co(trpn)ATP$^-$	none	0.80
	Co^{III}(trpn)(aq)	2700
	Co^{III}(cyclen)(aq)	1100
	Co^{III}(tn$_2$)(aq)	58
	Co^{III}(tren)(aq)	5.7
Co(tren)ATP$^-$	none	3.4
	Co^{III}(trpn)(aq)	820
	Co^{III}(tn$_2$)(aq)	37
	Co^{III}(tren)(aq)	10

The rate measurements we report are for the hydrolysis of ATP, in 1:1 cobalt to ATP complexes of type Co(N'_4)ATP$^-$, promoted by the addition of Co^{III}(N_4)(aq). The rates were measured in aqueous solution for pH 6.5 and 25 °C, with ionic strength 0.1 M ($NaClO_4$). The 1:1 complexes between Co^{III}(N'_4)(aq) and ATP (with N'_4 = trpn, cyclen, tren, or (tn)$_2$) were preformed in solution, as has been described in detail for the (tn)$_2$ and trpn systems.[2b,11,13] For these purposes Co^{III}(N'_4)(aq) was equilibrated with equimolar amounts of ATP at pH ~6.5; this procedure leads to almost quantitative production of Co(N'_4)ATP$^-$, existing predominantly as the β,γ-chelate, with very little accompanying hydrolysis of the ATP.[2b,11,13] The resulting 1:1 chelates exhibit very low reactivity toward hydrolysis of the ATP,[2b,11,13] and this process is of no importance here.

Solutions containing Co^{III}(N_4)(aq) and Co(N'_4)ATP$^-$ (each at an appropriate pH) were mixed, and while pH was monitored with time,[17] aliquots were quenched at various times with Eu^{2+} and H$^+$. Details on this method and the subsequent analysis for P_i have been given.[2b,13] We report the results of the initial rates ($v_0 = d[P_i]/dt$ [M s^{-1}]; see refs 13 and 18).

Results and Discussion

Initial rates of P_i production, following the addition of Co^{III}(N_4)(aq) to the preformed Co(N'_4)ATP$^-$, are summarized in Table I. General mechanistic features for the ensuing reactions have been elucidated,[2b,11,13,19] and detailed aspects have been presented where N_4 and N'_4 are (tn)$_2$ and/or trpn.[2b,13] The mechanism we have proposed is relatively straightforward when the reactant concentrations are equal to 10^{-3} M.[20] For these conditions the interpretation we have provided[2b] involves rapid substitution of the water in Co(N_4)(OH)(H_2O)$^{2+}$ to form a 2:1 (dinuclear) cobalt to ATP complex, where the Co^{III}(N_4)(OH) unit is linked to an oxygen on a γ- or α-phosphorus, facile rearrangement of the latter to provide a γ-linked Co^{III}(N_4)(OH) species,[21] and rate-determining attack by coordinated hydroxide on the γ-phosphorus (see Figure 1) to produce coordinated P_i (in competition with reactions leading to unreactive double chelates). Closely related mechanistic patterns have been proposed for the reactions of Co(tn$_2$)(OH)(H_2O)$^{2+}$ with Co(en$_2$)$P_2O_7^-$ and Co(tacn)(P_3O_{10})$^{2-}$ [22] and for the reaction of Co(cyclen)(OH)(H_2O)$^{2+}$ with Co(NH_3)$_4$$P_3O_{10}^{2-}$.[23]

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(3) Abbreviations: trpn = tris(3-aminopropyl)amine = N($CH_2CH_2CH_2NH_2$)$_3$; cyclen = 1,4,7,10-tetraazacyclododecane = HN(CH_2CH_2NH)$_3$$CH_2CH_2$; tren = tris(2-aminoethyl)amine = N($CH_2CH_2NH_2$)$_3$; tn = $H_2N(CH_2)_3NH_2$; N_4, (N'_4) = tetraamine ligand(s) occupying four coordination sites; "aq" in Co^{III}(N_4)(aq), Co^{III}(trpn)(aq), etc., refers to (H_2O)$_2$, (OH)(H_2O), or (OH)$_2$ depending on pH; ATP = adenosine 5′-triphosphate [the phosphate groups in ATP are labeled α, β, and γ, where the latter refers to the terminal phosphate group]; ADP = adenosine 5′-diphosphate, Ad = adenosine; P_i = orthophosphate; charges are often omitted for better readability.

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(20) At higher concentrations and higher Co^{III}(N_4)(aq) to Co(N'_4)ATP$^-$ ratios additional reaction paths develop; see ref 13.

(21) This rearrangement is analogous to the ring-opening step proposed in the reaction of (tn$_2$)CoATP$^-$ with added Cu(bpy)$^{2+}$; see ref 13.

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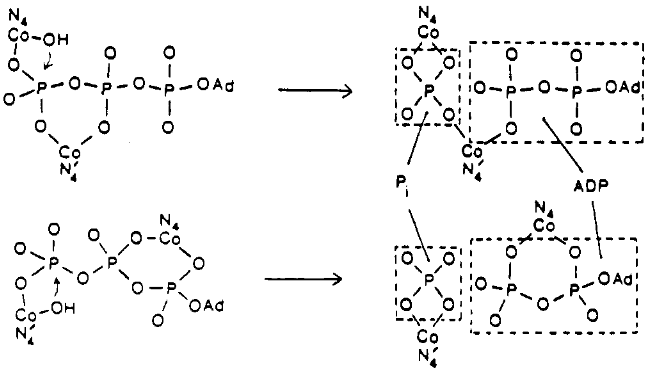

Figure 1. Elementary reactions leading to P_i and ADP (charges omitted).

The observed rates (Table I) are ***highly sensitive*** to the identity of N_4 (spread in relative v_0 values from $\sim 10^2$ to $>10^3$ for constant N'_4), and ***relatively insensitive*** to the identity of N'_4 (spread in relative v_0 values <10 for constant N_4); the order of reactivity for $Co^{III}(N_4)(aq)$, with constant $Co(N'_4)ATP^-$, is trpn > cyclen > $(tn)_2$ > tren. Thus with $N'_4 = (tn)_2$ the rate ratio is 1700 (N_4 = trpn):150 (cyclen):23 ($(tn)_2$):1 (tren), while with $N_4 = (tn)_2$, the rate ratio is only 2.5 ($N'_4 = tn_2$):1.6 (trpn):1 (tren). As is well established, the hydrolysis rates for each $Co(N'_4)ATP^-/Co^{III}(N_4)(aq)$ and $Co(N'_4)ATP^-/M^{n+}$ reaction exhibit a pH dependency.[11,13,24] However, if the $v_{0,max}$ values[25] for $Co(N'_4)ATP^-/Co^{III}(N_4)(aq)$ systems are compared for a given $Co(N'_4)ATP^-$ complex, the same order for N_4 dependency is seen, and the spread in relative $v_{0,max}$ values is almost as large as that seen for v_0 values at pH 6.5.[24] For example, for $N'_4 = (tn)_2$, where the pHs of maximum rates for N_4 = trpn, cyclen and $(tn)_2$ are 6.8, 7.2, and 6.5, respectively, the relative $v_{0,max}$ values still spread a 10^3-fold range.[25] These observations are consistent with limited earlier results on N_4/N'_4 ligand dependency for cobalt(III) promoted hydrolysis of ATP[2b,4a,19] and ADP[2a] and with related findings for several phosphate esters and polyphosphates.[2,4e,5a,5d,6]

The influences of the tetraamine ligands, N_4, on these phosphate hydrolysis rates bear a close resemblance to the influences of N_4 on substitution rates for complexes of the type *cis*-$Co^{III}(N_4)XY$. Thus, for aquation (acid hydrolysis[26]) reactions of *cis*-$Co(N_4)Cl_2^+$, the order of reactivity (at 25 °C) for the loss of the first chloride is also trpn > cyclen > tren;[4c,27-33] for loss of the second chloride the order is likewise trpn > tren[33] (data for cyclen not available) with the trpn complex reacting faster by a factor of 2×10^3. The marked influences of the N_4 ligands on substitution reactions of such complexes as *cis*-$Co(N_4)Cl_2^+$ have long been recognized.[16,34] Similar influences of N_4 on substitution of water in $Co(N_4)(OH)(H_2O)^{2+}$ ions are seen. Thus for phosphate anation at pH 6.5 (25 °C, I = 1.0 M) where N_4 = trpn, outer-sphere/inner-sphere phosphate interchange proceeds with $t_{1/2} \sim 1$ s, while for the trpn, $(tn)_2$ and *cis*-$(en)_2$ complexes the rates for this process are in the ratio 10^5:10^3:1.[4b]

The relative rapidity of water substitution in the hydroxoaqua ions $Co(N_4)(OH)(H_2O)^{2+}$ has contributed to the view that formation of the reactive 2:1 complexes $Co(N'_4)ATPCo(N_4)(OH)^+$, from $Co(N'_4)ATP^-$ and $Co^{III}(N_4)(aq)$, is not rate-limiting at the pHs where $Co(N_4)(OH)(H_2O)^{2+}$ predominates. For related systems the validity of this type of assumption has been questioned.[8] It has not been possible as yet to measure rates of formation for the reactive 2:1 complexes in these systems, or to establish their stabilities (formation constants) with respect to reactants, in part because of the ensuing hydrolysis (dephosphorylation) process. However, some insight can be gained into possible rates of formation for the reactive 2:1 complexes for our reaction conditions, by considering phosphate anation rates for the $Co(N_4)(OH)(H_2O)^{2+}$ ions.[4b] Thus we can estimate v_0 values for the anation of $Co^{III}(N_4)(aq)$ complexes {$Co(N_4)(OH)(H_2O)^{2+}$ major species} by P_i (orthophosphate), each at concentrations 10^{-3} M, for pH 6.5, 25 °C and I = 0.1 M (same conditions as for our present experiments). We obtain: N_4 = trpn, $v_0 = 2.9 \times 10^{-5}$ M^{-1} s^{-1}; $N_4 = tn_2$, $v_0 = 4.1 \times 10^{-7}$ M^{-1} s^{-1}. These rates are, respectively, 4.2 and 4.4 times the v_0 values observed (Table I) for our $Co(N'_4)ATP^-/Co^{III}(N_4)(aq)$ hydrolysis rates. For the latter systems, formation of the 2:1 complexes {from $Co(N'_4)ATP^-$ and $Co^{III}(N_4)(aq)$} could be somewhat slower, because of less favorable charge effects for outer-sphere complex formation, although the influence of triphosphate {chelated to one cobalt(III)} compared to P_i is hard to assess. This analysis, while incomplete, suggests that the rate-limiting step in the ATP hydrolysis is formation of the reactive 2:1 complex rather than the hydrolysis step itself.[35] The corollary, that attack of phosphorus by coordinated hydroxide in the 2:1 complex could be considerably more rapid than the rate of formation of the complex, has more general and important implications regarding the roles of metal ions in biological phosphoryl transfer.

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(31) The difference between the reactivities of the cyclen and tren complexes reported for 25 °C is small; also, for the cyclen complex, the two reported sets of activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$) are markedly different (see refs 27 and 28).

(32) *trans*-$Co(tn_2)Cl_2^+$ is omitted from this comparison because of its geometry; it undergoes rapid hydrolysis to the monoaqua chloro complex with a half-life of 13 s at 25 °C (see ref 16).

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(35) Caution should be used in extrapolating these arguments to related systems, such as the $Co(N_4)(OH)(H_2O)^{2+}$ promoted hydrolysis of orthophosphate esters. That the order of reactivity, for various N_4, is the same for substitution in *cis*-$Co(N_4)XY$ and for $Co(N_4)(OH)(H_2O)^{2+}$-promoted phosphate hydrolysis does not, *in itself*, demonstrate that the rate-limiting step in the hydrolysis process involves substitution at the Co^{III}. For both Co^{III} substitution and Co^{III}-promoted phosphate hydrolysis, significant reorganization of the cobalt coordination sphere must be required to achieve the transition state, and it seems likely that there would be closely related influences of the N_4 ligands on rates for the two classes of reactions.