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Communications

Adenosine 5′-Triphosphate (ATP) Hydrolysis Promoted by the Highly Reactive Hydroxoaquo(3,3′,3″-triaminotripropylamine)cobalt(III) Ion. Patterns of Reactivity and Mechanistic Considerations

Sir:

Metal-ion-promoted hydrolysis of ATP[1] and related polyphosphates and phosphate esters is of broad interest, especially in regard to the roles of metal ions in biological phosphoryl and nucleotidyl transfer[2,3] For model studies in this area some advantages can be realized by using complexes of cobalt(III).[4-10] We have previously examined the reactivity of free and bound ATP toward $(tn)_2Co^{III}(aq)$[5b,10] (tn = $H_2N(CH_2)_3NH_2$); at pHs where $(tn)_2Co(OH)(OH_2)^{2+}$ predominates, this aquo complex promotes ATP hydrolysis at rates as high as reported for any aquo metal ion.[10,11] We now describe reactions of the much more reactive $trpnCo(OH)(OH_2)^{2+}$ with free and complexed ATP as well as subsequent reactions of the complexes formed (trpn = $N(CH_2CH_2CH_2NH_2)_3$). The extraordinary reactivity patterns observed for the $trpnCo^{III}$/ATP systems, which include rate accelerations in excess of 10^6 for ATP hydrolysis as well as dramatic rate saturation effects, together with comparisons with the $(tn)_2Co^{III}$/ATP systems, highlight some of the important factors influencing rates and mechanisms for reactions of this type.

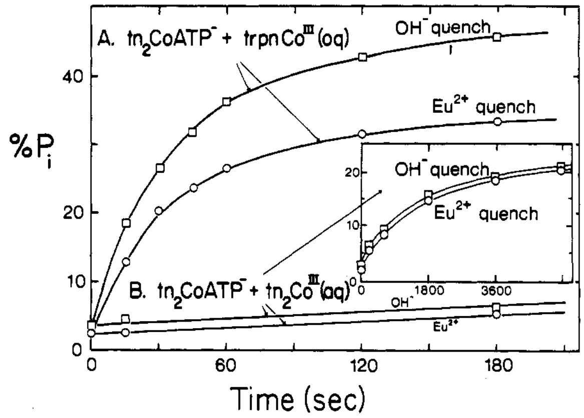

Figure 1. Hydrolysis of $(tn)_2CoATP^-$ at 2:1 Co to ATP ratio (i = initial, av = average): (A) $(tn)_2CoATP^-$ (10^{-3} M) + $trpnCo^{III}(aq)$ (10^{-3} M), pH_i 6.6 (pH_{av} 6.6, 25 °C, I = 0.1 M); (B) $(tn)_2CoATP^-$ (10^{-3} M) + $(tn)_2Co^{III}(aq)$ (10^{-3} M), pH_i 6.4 (pH_{av} 6.7, 25 °C, I = 0.1 M).

The 1:1 complexes $(tn)_2CoATP^-$ and $trpnCoATP^-$, existing predominantly as the β,γ chelates (Figure 2, I), were prepared in solution, in a modification[10] of a previous procedure,[5b] by addition of a solution containing $N_4Co^{III}(aq)$ (pH adjusted to 6.5) to a solution containing an equimolar amount of ATP (pH also 6.5). The pH, which rose rapidly due to complex formation, was adjusted with $HClO_4$ (glass stick, dotting) until it was steady at 6.5 (achieved in time of mixing for the trpn system and in ~2 min for the $(tn)_2$ system). During formation of I a small amount of ATP becomes hydrolyzed to ADP and P_i (see below). The six-membered chelates I exhibit very low reactivity toward hydrolysis of the ATP,[5b,10] and this process is not of importance here.

To solutions containing the preformed $(tn)_2CoATP^-$ or $trpnCoATP^-$, equimolar amounts of $(tn)_2Co^{III}(aq)$ or $trpnCo^{III}(aq)$ were added to provide solutions at 25 °C with initial pHs of ~6.5 ($N_4Co(OH)(OH_2)^{2+}$ is the predominant form for the latter complexes[12,13]). While the pH was monitored with time,[14] aliquots were quenched at various times by two separate methods, (1) with Eu^{2+} and H^+ and (2) with OH^- (2 M after quenching), and after further treatment (below) these were analyzed for P_i by an established method based on phosphomolybdate.[15] In method 1,

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[1] ATP, ADP, and AMP = adenosine 5′-tri-, 5′-di-, and monophosphates; P_i = orthophosphate.

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[11] In a comparison of the effectiveness of different metal ions, caution is necessary because of differing dependencies of rates on concentration and pH (discussed fully in ref 10).

[12] (a) Jonasson, I. R.; Lincoln, S. F.; Stranks, D. R. *Aust. J. Chem.* **1970**, *23*, 2267–2278. (b) Fowless, A. D.; Stranks, D. R. *Inorg. Chem.* **1977**, *16*, 1276–1281.

[13] Massoud, S. S.; Milburn, R. M., unpublished results.

[14] For most experiments buffers were not used to avoid influences on reaction rates (see: Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5*, 467–477. Amsler, P. E.; Sigel, H. *Eur. J. Biochem.* **1976**, 63, 569–581). When the experiments with preformed $(tn)_2CoATP^-$ and added $(tn)_2Co(OH)(OH_2)^{2+}$ or $trpnCo(OH)(OH_2)^{2+}$ were repeated, with the pH maintained at 6.5 with use of 1.5 × 10^{-2} M BIS-TRIS buffer, the rates were ~30% lower than before, but the reaction patterns (including rate saturation) were otherwise very similar to those observed before.

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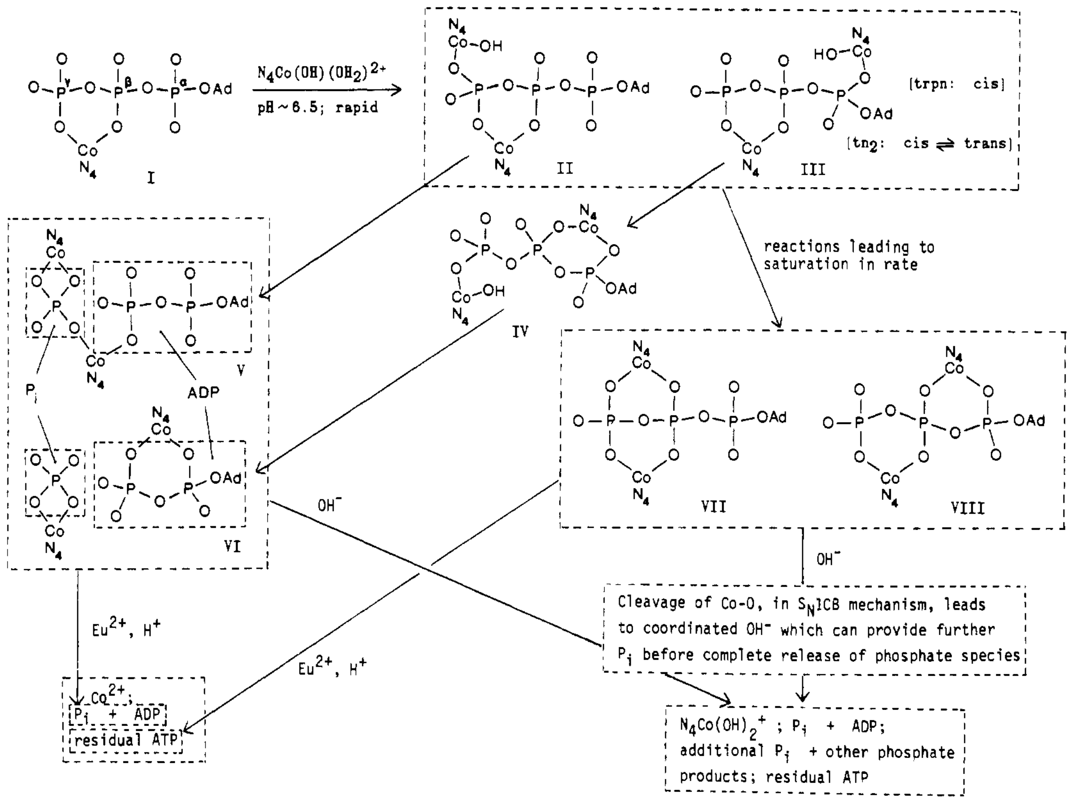

Figure 2. Suggested features for overall mechanisms (charges mostly omitted).

an excess of Eu^{2+} and H^+ (Cl^-) was added to reduce the Co(III) to Co(II) and to release all bound phosphate species; the remaining Eu^{2+} was then air oxidized to Eu^{3+} (process achieved in less than 1 min[16]), and the analysis for P_i was carried out immediately.[17] In method 2, used previously,[5b,10] the quenched solutions were left for ~12 h to release all bound phosphate before acidifying with $HClO_4$ and immediately analyzing for P_i. Values of percent P_i are in reference to complete conversion of ATP to ADP and P_i.

The analysis following Eu^{2+} quenching (method 1) gives P_i initially present in the ATP (0.7%) together with that formed during production of the 1:1 complex ($(tn)_2CoATP^-$, 1.6%; $trpnCoATP^-$, 5.9%) and that produced in further reactions with $N_4Co^{III}(aq)$; analysis for P_i following OH^- quenching (method 2) adds to the above that produced during complex destruction by OH^-.

Figure 1 summarizes results for the reactions of $(tn)_2CoATP^-$ with (A) $trpnCo^{III}(aq)$ and (B) $(tn)_2Co^{III}(aq)$. A closely related set of results was obtained for the reactions of $trpnCoATP^-$ with (A) $trpnCo^{III}(aq)$ (rapid initial rates followed by rate saturation) and (B) $(tn)_2Co^{III}(aq)$ (much lower initial rates, also followed by rate saturation).[13] For the reaction of $(tn)_2CoATP^-$ with $trpnCo^{III}(aq)$, the observed initial rate of P_i production (Figure 1A, slope, Eu^{2+} quenching method) corresponds to an ATP hydrolysis rate of 7×10^{-6} M s^{-1}, which represents an enhancement of $\sim 1 \times 10^6$ over the unpromoted hydrolysis rate for ATP at the same temperature (25 °C) and pH (~6.5).[5b] By suitably modifying the concentration conditions, one can obtain significantly larger rate enhancements.[18]

The major observations of this study are then as follows:

(1) Reaction of the β,γ chelates N_4CoATP^- (N_4 = trpn or $(tn)_2$) with $trpnCo^{III}(aq)$ at pH ~6.5, where $trpnCo(OH)(OH_2)^{2+}$ predominates, results in (a) very high initial rates for ATP hydrolysis ($\sim 1 \times 10^6$ times the unpromoted rate, at 10^{-3} M reactant concentrations and 25 °C),[18] (b) marked decreases in rate with time (rate saturation),[19] and (c) significant yields of additional P_i on OH^- quenching.

(2) Reaction of the β,γ chelates N_4CoATP^- (N_4 = trpn or $(tn)_2$) with $(tn)_2Co^{III}(aq)$ at pH ~6.5, where $(tn)_2Co(OH)(OH_2)^{2+}$ predominates, results in (a) lower initial rates for ATP hydrolysis ($\sim 1 \times 10^4$ times the unpromoted rate, at 10^{-3} M reactant concentrations and 25 °C),[18,20] (b) comparably marked decreases in rate with time (rate saturation) displayed over a correspondingly longer time span,[19] and (c) low yields of additional P_i on OH^- quenching, especially for $(tn)_2CoATP^-$.

These observations can be understood in mechanistic terms, summarized by Figure 2, which relate to rapid formation of the monodentate complexes II and III (arising from the unusually rapid rates of water substitution in $(tn)_2Co(OH)(OH_2)^{2+}$ [12,13] and especially in $trpnCo(OH)(OH_2)^{2+}$ [5c,13]), the cis arrangement of the oxygen donor ligands attached to the $trpnCo^{3+}$ center (dictated by the tripodal nature of trpn, which gives rise to appropriately positioned coordinated hydroxide), the cis ⇌ trans interconversions characteristic for the $(tn)_2Co^{III}(O)(O)$ systems (where cis but not trans geometry provides an appropriately positioned cooridinated hydroxide), and incorporation of the second cobalt into new chelate rings in VII and VIII (which gives rise to the rate saturation[21]). High proportions of the $(tn)_2$ trans isomers for II and III, and sluggish rates for trans → cis isomerizations in these complexes, could be important factors in the lower reactivity of $(tn)_2Co(OH)(OH_2)^{2+}$ compared to that of $trpnCo(OH)(OH_2)^{2+}$.[20]

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(17) No significant differences were observed when Eu^{3+} and Co^{2+} were first removed by cation exchange (H^+). This feature provides the Eu^{2+} method with an advantage over the V^{2+} quenching method[7] since the vanadium must be removed because of its strong interference with the phosphomolybdate analysis. Also, vanadium interacts strongly with the N-7 of the adenine ring (Mikulski, C. M.; Cocco, S.; de Franco, N.; Karayannis, N. M. *Inorg. Chim. Acta* **1983**, *80*, L61–L64) and for the present system we were unable to separate vanadium from the reaction mixtures by cation exchange.

(18) Rate enhancements become significantly larger for $[N_4Co^{III}(aq)]/[N_4CoATP^-] > 1$ and for $[N_4CoATP^-] > 10^{-3}$ M.[5b,10,11]

(19) P_i production falls off much more rapidly than would be the case if the reaction were second order in ATP.

(20) The slightly differing dependencies of rates on pH, for the $(tn)_2$ and trpn systems, is not an important factor in the large differences in rates at pH ~6.5.

(21) Possible development of the $\beta\gamma,\alpha\gamma$ double chelate has also been considered, but with $trpnCo^{3+}$ $\alpha\gamma$ chelate formation is sterically difficult, as may be seen with use of space-filling models. (One of the reviewers drew our attention to this feature.)

Similar studies[22] in which (4,11-CT)Co(OH)$(OH_2)^{2+}$ (4,11-CT = Me_6[14]diene(4,11)N_4) was added to preformed β,γ-$(tn)_2CoATP^-$ led to no observable enhancements in rates of P_i production (except for the very slight enhancements characteristic for the latter complex alone). In (4,11-CT)Co(OH)$(OH_2)^{2+}$ the water and hydroxide are exclusively trans to one another. The additional P_i, formed after OH^- quenching, can arise through a conventional S_N1CB mechanism[6] in which ring opening of a diphosphate chelate (in VI, VII, and VIII) or loss of a monodentate coordinated phosphate (in V) provide cis-coordinated hydroxide. A preference for trans geometry following the initial Co–O cleavage would explain the low yields of additional P_i where the $(tn)_2Co^{III}$ moiety is involved.[23] The latter aspects are under continued study.

The present studies, augmenting earlier findings from several laboratories, provide a clear demonstration of mechanistic features that should be of general importance in the promotion or inhibition of phosphoryl-transfer reactions by aquo metal ions: these include the requirement of cis-coordinated OH^- for high reactivity in the middle pH region,[3,5–7] the importance of cis/trans isomerism at the metal center,[6b–d,7] the importance of complexes containing more than one metal ion for effective promotion of polyphosphate hydrolysis,[3–5,6d,7] and the property of low reactivity for complexes in which all constituent metal ions are saturated by full chelation.[4,5,6d,8] Especially noteworthy, for the present systems, are the high initial rates for P_i production on addition of trpnCo(OH)$(OH_2)^{2+}$ to β,γ-$N_4Co^{III}ATP^-$, the marked falloff in rates attributable to competitive reactions in which the added aquo metal ions become saturated by full chelation, and demonstration of the advantages of the Eu^{2+} quenching method for release of phosphate species coordinated to cobalt(III). The mechanistic features displayed have relevance to enzyme-promoted phosphoryl-transfer and related processes with metal ion requirements; here additional steric constraints imposed by the enzyme system may be expected to have marked influences on isomeric possibilities for metal complexes and hence on the manner in which metal ions and coordinated nucleophiles can participate.

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Registry No. ATP, 56-65-5; trpnCo(OH)$(OH_2)^{2+}$, 96914-52-2; $(tn)_2CoATP^-$, 96914-53-3; trpnCo^{III}(aq), 96914-54-4; $(tn)_2Co^{III}$(aq), 89921-37-9; trpnCoATP⁻, 96914-55-5.

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[23] From Figure 1B one can see that quenching with OH^- produces only 1–2% additional P_i, with this amount independent of the quenching time.

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Characterization of an Easily Obtainable Palladium(IV)-Amidate Complex

Sir:

Transient palladium(IV) intermediates have been invoked to explain certain palladium-catalyzed transformations of organic compounds;[1] however, the paucity of palladium(IV) complexes with adequate stability in solution has hampered a detailed study of palladium(IV) chemistry.[2–4] Here we report on the electrochemical preparation of a palladium(IV)–amidate complex that has sufficient stability in the absence of other strong oxidants in aqueous and nonaqueous solvents for the ready characterization of its properties and for the sampling of its reactivity.

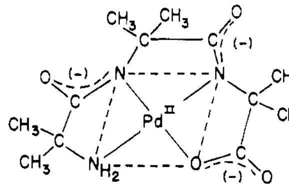
Table I. Spectral Data for Palladium(II, IV)–Aib_3 Complexes

species	medium conditions	λ_{max}, nm (ϵ, M^{-1} cm^{-1})	^1H NMR[a] δ_{CH_3}
$H_2Aib_3^+$	"pH" = 3[b]	<210	1.41 1.25 1.24
1	0.10 M $NaClO_4$	<250 292 (1400)	1.30 1.20 1.11
2	0.10 M NaCl[c]	243 (27 500) 300 (7500) sh 390 (1500) sh 494 (660)	1.53 1.47 1.32
2	0.10 M NaBr[c]	266 310 sh 405 sh 522	
2	0.10 M NaCl[d] or $NaClO_4$[d]	233 sh 275 sh 430 sh	

[a] D_2O solution. [b] The "pH" of a D_2O solution. [c] 0.050 M chloroacetate, pH 3.0. [d] 0.050 M phosphate, pH 7.0.

The ligand system that stabilizes the palladium(IV) state is the tripeptide of α-aminoisobutyric acid, H–[NHC$(CH_3)_2$CO]$_3$–OH, $HAib_3$. The donor atoms responsible for this stabilization are anionic deprotonated amide nitrogens, i.e. amidate nitrogens. Multidentate ligands with amidate nitrogen donors have been used to characterize uncommon, high-valent states for other transition metals, e.g. copper(III),[5] nickel(III),[5c] silver(III),[6] osmium(VI),[7] and cobalt(IV)[8].

Cyclic voltammetry indicates that the electrochemical oxidation of $[Pd(H_{-2}Aib_3)]^-$[9] (**1**), the precursor to the palladium(IV)



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[9] **1** was prepared by combining aqueous solutions of $HAib_3$ (10% excess) and $[PdCl_4]^{2-}$, slowly raising the pH to 7, and stirring at room temperature for several hours. After this time the spectrum for $[PdCl_4]^{2-}$ disappeared, and the spectrum for fully formed **1** was obtained. Circular dichroism[10,11] and UV–vis[10,12] spectral measurements and potentiometric titration[10] have shown that the absorption at 292 nm (see Table I) is characteristic of palladium(II)–tripeptide complexes in solution when the central metal ion is coordinated by an amine nitrogen, two amidate nitrogens, and a carboxylate oxygen. ^1H NMR spectra were collected on a Varian FT-80 instrument. The EPR spectra were recorded on a Varian E-3 spectrometer. Electronic spectra were obtained on a Perkin-Elmer Model 330 UV-VIS-NIR spectrophotometer.

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