# Comparison of the Effectiveness of Various Metal Ions on the Promoted Dephosphorylation of Adenosine 5'-Triphosphate (ATP) and Uridine 5'-Triphosphate (UTP)[2]

**Ronald M. Milburn,*[1b] Mamta Gautam-Basak,[1a] Roger Tribolet,[1a] and Helmut Sigel*[1a]**

*Contribution from the Institute of Inorganic Chemistry, University of Basel,[1a] CH-4056 Basel, Switzerland, and the Department of Chemistry, Boston University,[1b] Boston, Massachusetts 02215.* *Received July 3, 1984*

**Abstract:** Initial dephosphorylation rates ($v_0$ = d[$PO_4$]/d$t$) for ATP and UTP (=NTP) have been measured in $10^{-3}$ M solutions at pH 5.5 and 7.5 at 50 °C ($I$ = 0.1, $NaClO_4$), and the promotional influences of $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Cu^{2+}$, Cu(2,2'-bipyridyl)$^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $(tn)_2Co^{III}$ (tn = 1,3-diaminopropane), $Y^{3+}$, and $La^{3+}$ (=$M^{n+}$) have been compared. In most cases the $M^{n+}$/NTP 2:1 mixture is considerably more reactive than the 1:1 mixture, in accord with earlier Job's series with $M^{2+}$/NTP systems which showed that the reactive species contains two metal ions per nucleotide. A Job's series for the $(tn)_2Co^{III}$/ATP system indicates now, also in agreement with earlier suggestions, that in this case, 2:1 and 3:1 complexes are highly reactive. Experiments repeated for $10^{-2}$ M NTP confirmed that for the divalent metal ions $v_0$ is proportional to $[(M^{2+})_{1or2}/UTP]$ and also to $[(M^{2+})_{1or2}/ATP]^2$, i.e., with UTP the most reactive species is a monomeric $[M_2(UTP)](OH)^-$, while with ATP it is a dimeric $[M_2(ATP)]_2(OH)^-$ (details in Sigel et al., ref 4). With $(tn)_2Co^{III}$ $v_0$ is proportional to $[((tn)_2Co^{III})_{1or2}/ATP$ or $UTP]^{1.6}$; it is suggested that this result is due to increasing contributions to reactivity by a 3:1 Co(III) to NTP complex as reactant concentrations are increased. The difficulties and "pitfalls" in making general statements about the relative promotional effects of the metal ions, because of the differing dependencies of rates on concentration and pH, are illustrated and summarized. However, divalent metal ions with a coordination tendency toward N-7 of ATP promote the dephosphorylation of ATP better than of UTP; this is connected with a metal ion bridge within the $[M_2(ATP)]_2(OH)^-$ dimer. Each of the three trivalent metal ions promotes the dephosphorylation to the same extent in ATP and UTP; here, where no metal ion/base interaction occurs, the charge on these ions has much to do with their effectiveness. Overall one may say that the addition of $Cu^{2+}$, $(tn)_2Co^{III}$, $Y^{3+}$, or $La^{3+}$ to NTPs leads to systems which are especially reactive, while systems involving only $Mg^{2+}$ are especially unreactive. Several mixed metal ion/ATP systems have been studied, and these can also lead to marked promotion of reactivity.

Metal ions are essential to biological phosphoryl and nucleotidyl transfer,[7-10] and this feature continues to provide impetus to investigations of metal ion promoted phosphoryl-transfer reactions in model systems. Nucleoside di- and triphosphates, especially ATP,[11] were used as substrates. Related studies have focused on simple polyphosphates and on orthophosphate esters.

Previous work[12-16] on the influence of divalent metal ions on the dephosphorylation[11d] of ATP and other nucleoside 5'-triphosphates (NTPs) has provided some comparisons for various metal ions, as well as insight into the nature of the reactive species,[3,4,16] with $Cu^{2+}$ being particularly effective.[4]

Metal centers of other oxidation states have also been examined. Thus, early studies demonstrated that lanthanide ions and/or lanthanide hydroxide gels can be good promoters for the dephosphorylation of ATP,[17-19] as well as for the hydrolysis of phosphate esters.[20] More recent work has shown that certain Co(III) complexes can also be very effective in promoting ATP[5,21,22] and ADP[6,23] dephosphorylation, in addition to the hydrolysis of pyrophosphates,[24] triphosphate,[25] and orthophosphate esters.[23,26] Large enhancements in ATP dephosphorylation rates have also been reported for $VO_2^+$ and for $VO^{2+}$ in the presence of oxidants.[27]

(1) (a) University of Basel. (b) Boston University.
(2) (a) This is part 9 in the series "Hydrolysis of Nucleoside Phosphates" (published by H. S.); for parts 7 and 8, see ref 3 and 4, respectively. (b) For related papers (published by R.M.M.), see ref 5 and 6.
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(11) Abbreviations and definitions: (a) ADP or ATP, adenosine 5'-di-or -triphosphate; bpy, 2,2'-bipyridyl; CTP, cytidine 5'-triphosphate; en, ethylenediamine = 1,2-diaminoethane; $M^{n+}$, metal ion; NDP or NTP, nucleoside 5'-di- or -triphosphate; R-TP, triphosphate with one terminal and non-coordinating organic residue; tn, trimethylenediamine = 1,3-diaminopropane; UTP, uridine 5'-triphosphate. (b) The phosphate groups in NTPs are labeled $\alpha$, $\beta$, and $\gamma$, where the latter refers to the terminal phosphate group (see Figure 1). (c) If nothing else is specified, the formula $PO_4$ represents all related species which may be present in solution, i.e., $H_3PO_4$, $H_2PO_4^-$, $HPO_4^{2-}$, and $PO_4^{3-}$. Also, charges are sometimes omitted for better readability; e.g., $(tn)_2Co^{III}$ refers to any of the species $(tn)_2Co(OH_2)_2^{3+}$, $(tn)_2Co(OH)(OH_2)^{2+}$, or $(tn)_2Co(OH)_2^+$. (d) The term "dephosphorylation" refers to the transfer of a phosphoryl group to a water molecule; the term "hydrolysis" can also refer to this or a closely related process, but for the most part we use "hydrolysis" in reference to the formation of hydroxo complexes of metal ions. (e) The terms monomeric or dimeric mean that one or two $NTP^{4-}$ are in the complex, together with at least the corresponding number of $M^{n+}$; hence, e.g., $M_2(NTP)$ is a monomeric (but dinuclear) nucleotide complex whereas $[M(NTP)]_2^{4-}$ is a dimeric complex.
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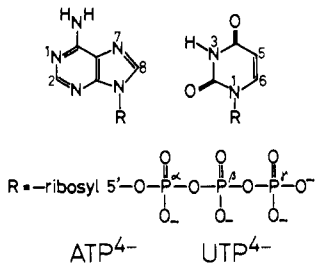


**Figure 1.** Structures of adenosine 5′-triphosphate ($ATP^{4-}$) and uridine 5′-triphosphate ($UTP^{4-}$), together with the labeling system for the triphosphate chain.

However, as the experiments with different metal ions have mostly been carried out under different conditions, conflicting views exist in the literature regarding the effectiveness of various metal ions in the promotion of NTP dephosphorylation. Therefore, the object of the present study is to provide a comparative analysis for the metal ion promoted dephosphorylations of NTPs. We have carried out all experiments under identical conditions ($I$ = 0.1, $NaClO_4$; 50 °C), and by quantifying the extent of dephosphorylation with use of the initial rates for phosphate liberation, i.e., $v_0 = d[PO_4]/dt$ ($M\ s^{-1}$), we are using an evaluation method which is independent of the order of the reaction (e.g., be it first or second) and the reaction mechanisms.

To obtain a solid basis for comparisons, we chose ATP as the characteristic example of the purine-NTPs, and UTP as a representative of the pyrimidine-NTPs (Figure 1), because it is known that the dephosphorylation of purine- and pyrimidine-NTPs is promoted by divalent metal ions in different ways.[3,4,16] To get some insight into the requirements of the reactions, we varied the pH as well as the total and relative concentrations of the NTPs and the metal ions. In particular, we compare the effects of some selected divalent (Mg, Mn, Ni, Cu, Zn, and Cd) and trivalent metal ions (Co, Y, and La); special attention is given to $(tn)_2Co^{III}(aq)$ [tn = $H_2N(CH_2)_3NH_2$; aq = $(H_2O)_2$, $(H_2O)(OH^-)$, or $(OH^-)_2$], a complex which has previously been shown[5,6,24,26] to be very effective in facilitating reactions of this type. In addition, several mixed metal ion/ATP systems have been studied to evaluate the possibility of synergistic effects between metal ions.

## Experimental Section

**Materials.** The disodium salt of ATP (>98%; p.a.) and the trisodium salt of UTP (research grade) were from Serva Feinbiochemica GmbH, Heidelberg, FRG. Stock solutions, after pH adjustment to ~8.5, were stored at 0–3 °C and used within a few days; the initially present free $PO_4$ was determined daily.

$Cd(ClO_4)_2$ was purchased from Ventron GmbH, Karlsruhe, FRG, and $Zn(ClO_4)_2$ from K&K Laboratories, Cleveland, OH. All the other metal(II) perchlorates were from Fluka AG, Buchs, Switzerland. The concentrations of their stock solutions were determined by titrations with EDTA (Merck AG). $NaClO_4$, $Y(NO_3)_3 \cdot 5\ H_2O$, and $La(NO_3)_3 \cdot 6\ H_2O$ were obtained from Merck AG, Darmstadt, FRG.

Solutions containing $(tn)_2Co^{III}$ were prepared by dissolving *trans*-$[(tn)_2Co(OH)(OH_2)](ClO_4)_2$ in water.[11c] The latter complex, which was shown by elemental analysis to be anhydrous, was prepared as before,[26a] from $[(tn)_2CoCO_3]ClO_4$ following the method of Stranks et al.[28] Solutions of the hydroxoaqua complex were stored in the refrigerator.

**Measurement of Rates.** Apart from the modifications described below, the dephosphorylation, which refers to the conversion of NTP to NDP and $PO_4$,[5,13] was followed by procedures already described,[3,4,15,16,29] and the initial rate was determined: $v_0 = d[PO_4]/dt$ ($M\ s^{-1}$) (see ref 15 and 29). The concentrations of free $PO_4$ were measured with molybdate reagent, in samples taken in suitable intervals, using an adaption[14] of the method developed by Hirata and Appleman.[30] The absorption measurements were made at 328 nm with Bausch & Lomb Spectronic 88 and Varian Techtron 635 spectrophotometers. Measured rates for the dephosphorylation were at 50 °C (±0.2°) and an ionic strength of 0.1 M (adjusted with $NaClO_4$). Buffers were not used to avoid influences on reaction rates;[15] the pH was adjusted with NaOH or $HClO_4$ by using a glass stick ("dotting"; the volume changes were negligible). Usually $v_0$ is reproducible to ±10%, but for very rapid reaction and/or an unstable pH, the error limit may increase to about ±25%.

In studies with $(tn)_2Co^{III}$, aliquots of the reaction solution were quenched at various times by addition to equal volumes of 4 M NaOH; these quenched solutions were then held in the dark at room temperature for a constant time (about 10–14 h) during which all orthophosphate, NDP, and residual NTP are released from the Co(III) center.[5,6,24] In an independent experiment, the half-life for $PO_4$ release from $(tn)_2CoPO_4$ at ~20 °C was shown to be ~55 min. The dephosphorylation of the NTPs was in most cases followed for 20–30% reaction, with data covering the first 10% reaction being the most useful for the calculation of $v_0$. The quenching procedure leads to no significant further dephosphorylation of the NTPs for any of the systems reported. The remainder of the analytical procedure was as described above, except that sufficient additional $HClO_4$ was included in the $HClO_4$/molybdate solution to neutralize the strong base, and the heat generated from the strong acid/strong base reaction was compensated for by holding the solutions, after addition of ice-cold acetone and volume adjustment, for 2 min in a 19 °C bath rather than a 25 °C bath prior to spectrophotometric measurements. The results obtained in this way with molybdate reagent are in good agreement with earlier measurements using $^{31}P$ NMR or an enzymic method.[5]

For all rate measurements with $(tn)_2Co^{III}$, the 1:1 complex of ATP or UTP was preformed as follows: To a solution of the NTP, at pH 6.5 and room temperature, an equimolar amount of $(tn)_2Co^{III}$ solution was added with stirring during ~1 min. The pH, which rose rapidly due to inner-sphere complex formation, was readjusted to ~6.5 by dotting with $HClO_4$. Complex formation is established within a further 2 min as seen by achievement of a steady pH. The solution was then transferred to a 50 °C water bath, and the pH was readjusted to 6.5; equilibration was then continued for 10 min. The resulting $(tn)_2Co(NTP)^-$ complex, which is formed essentially quantitatively,[5] was then used, after any necessary further pH adjustment, for studying the dephosphorylation in the 1:1 ratio, or in the 2:1 cobalt-to-NTP ratio upon addition of a second equivalent of $(tn)_2Co^{III}$. For the 2:1 ratio, preforming the 1:1 complex (assisted by pH control) minimized the time required for preequilibration. For the mixed metal ion systems (Table III) the second metal ion was likewise added to the preformed $(tn)_2Co(NTP)^-$ complex.

## Results and Discussion

**1. Some Established Results for the Promoted Dephosphorylation of NTPs by Divalent Metal Ions.** It will be helpful to review shortly some of the more important features of the acceleration of the dephosphorylation of pyrimidine-NTPs and purine-NTPs by divalent metal ions which have recently been established.[3,4,16] and described in detail.[4]

(i) The most reactive species contains two metal ions per NTP, one coordinated to the terminal γ phosphate and the other to the α,β groups. The nucleophilic attack at the γ phosporus occurs in an intramolecular fashion via a $M\text{–}OH^+$ unit, though under certain conditions an intermolecular attack by water is also possible.

(ii) All pyrimidine-NTPs show the same properties in the dephosphorylation as simple triphosphate esters, such as methyl triphosphate; i.e., the nucleic base moiety has no influence on the reaction rate (up to pH ~8), whereas the purine-NTPs differ among each other, and with certain metal ions, like $Cu^{2+}$, the dephosphorylation is much more accelerated than for the pyrimidine-NTPs. This larger dephosphorylation rate is achieved by the formation of a dimeric species via base-stacking and by the facilitated formation of the reactive state in this dimer via a $M^{2+}$/N-7 interaction; inhibition of this interaction, e.g., by addition of 1 equiv of 2,2′-bipyridyl, leads therefore to an inhibition of the reactivity (Table I).

(iii) The most reactive species is a $M_2(R\text{–}TP)(OH)^-$ complex for the pyrimidine-NTPs and a $[M_2(NTP)]_2(OH)^-$ complex for ATP and (in part) other purine-NTPs.

**2. Influence of Metal Ion Excess on the Rates of NTP Dephosphorylations.** The observed dephosphorylation rates for $10^{-3}$ M solutions of ATP and UTP are summarized (as $v_0 \times 10^8$ M $s^{-1}$) for the $M^{n+}$/NTP ratios of 1:1 and 2:1 at pH 5.5 and 7.5 in Table I. It is evident that for practically all systems, the 2:1 ratio

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**Table I.** Comparison of the Initial Rate of Dephosphorylation, $v_o$ (M $s^{-1}$), of Nucleoside 5′-Triphosphates (NTP) in $10^{-3}$ M Solutions in the Presence of Different Metal Ions ($I$ = 0.1, $NaClO_4$; 50 °C). All Initial Rates Are Given as $v_o \times 10^8$

| | | [$M^{n+}$]/[NTP] (1:1) | | [$M^{n+}$]/[NTP] (2:1) | |
|---|---|---|---|---|---|
| NTP | $M^{n+}$ | pH 5.5 | pH 7.5 | pH 5.5 | pH 7.5 |
| ATP | none[a] | 0.11 | 0.032 | 0.11 | 0.032 |
| | $Mg^{2+}$ | 0.096 | 0.045 | 0.11 | 0.050 |
| | $Mn^{2+}$ | 0.10[b] | 0.060[b] | 0.2 | 0.15 |
| | $Ni^{2+}$ | 0.075[b] | 0.05[b] | 0.1[c] | 0.14[c] |
| | $Cu^{2+}$ | 2.5[c] | 7.5[c] | 30[c] | 7.5[c] |
| | $Cu(bpy)^{2+}$ | 0.060[c] | 0.052[c] | | |
| | $Zn^{2+}$ | 0.15[b] | 0.25[b] | 0.5[c] | 1.2[c] |
| | $Cd^{2+}$ | 0.15[d] | 0.25[d] | 0.7[d] | 3.2[d] |
| | $(tn)_2Co^{III}$ | 0.45 | 0.16 | 130 | ~80 |
| | $Y^{3+}$ | 2[e] | ~23[f] | ~8[g] | ~70[g] |
| | $La^{3+}$ | 2[e] | 23[f] | ~7[g] | 70[g] |
| UTP[h] | none[i] | 0.11 | 0.032 | 0.11 | 0.032 |
| | $Mg^{2+}$ | 0.061 | 0.038 | 0.062 | 0.041 |
| | $Mn^{2+}$ | 0.10 | 0.052 | 0.1 | 0.09 |
| | $Ni^{2+}$ | 0.065[j] | 0.046[j] | 0.064 | 0.057 |
| | $Cu^{2+}$ | 0.17[j] | 0.56[j] | 0.7[j] | g |
| | $Cu(bpy)^{2+}$ | 0.060[j] | 0.052[j] | | |
| | $Zn^{2+}$ | 0.08[j] | 0.09[j] | 0.1 | ~0.7[g] |
| | $Cd^{2+}$ | 0.1 | 0.1 | 0.13 | ~0.8[g] |
| | $(tn)_2Co^{III}$ | 0.45 | 0.16 | 120 | ~65 |
| | $Y^{3+}$ | ~2[f] | ~23[f] | ~8[g] | ~70[g] |
| | $La^{3+}$ | 2[e] | 23[f] | 7[g] | ~70[g] |

[a] From ref 3, 4, 15, and 16. [b] From ref 15. [c] From ref 16. [d] From ref 4. [e] The solution appeared slightly "milky", but there was no clear sign of a precipitate. [f] The solutions for these experiments were completely clear. [g] Precipitation. [h] The same results are obtained also with CTP for the entries: none (cf. ref 15, 16), $Ni^{2+}$ (cf. ref 15), $Cu^{2+}$ (cf. ref 16), $Cu(bpy)^{2+}$ (cf. ref 16), $Zn^{2+}$ (cf. ref 15), and $Cd^{2+}$ (cf. ref 4). [i] From ref 3 and 4. [j] From ref 3.

is more effective in promoting the reaction than the 1:1 ratio, the only clear exception being $Cu^{2+}$/ATP which at pH 7.5 shows the same reactivity for both ratios.

These findings agree with earlier studies[3,4,16] of $M^{2+}$/NTP systems for which Job's series showed throughout a 2:1 composition for the reactive species, including $Cu^{2+}$/ATP at pH <6.5;[16] for $Cu^{2+}$/ATP at pH >6.5, one $Cu^{2+}$ simply hydrolyzes away and can therefore no longer promote the dephosphorylation reaction.[4,16]

The present results for the $(tn)_2Co^{III}$/ATP system are also in accord with a previous study[5] which indicated that reactive complexes containing two and three metal centers per ATP are developed (vide infra; section 5). Similarly, the results (Table I) obtained with $(tn)_2Co^{III}$/UTP and $Y^{3+}$- or $La^{3+}$/NTP support the view, despite the experimental difficulties experienced with $Y^{3+}$ and $La^{3+}$, that effective metal ion promotion of NTP dephosphorylation requires the formation of species involving more than one metal ion per NTP.

**3. Comparison of the Dephosphorylation Rates in Several Metal Ion/NTP Systems and Importance of Hydroxo Complex Formation.** The data in Table I evidence that at pH 5.5 and 7.5, only a few metal ions are really effective promoters of the dephosphorylation of ATP and UTP. For example, at pH 7.5 in a 1:1 ratio, $Cu^{2+}$ facilitates the ATP dephosphorylation by a factor of about 230 and $Y^{3+}$ or $La^{3+}$ that of ATP or UTP by factors of about 700. Similarly, at a 2:1 ratio and pH 7.5 the rates increase for the $(tn)_2Co^{III}$, $Y^{3+}$- or $La^{3+}$/ATP or -UTP systems by factors of 2000–2500. In contrast, $Mg^{2+}$ does practically not facilitate the dephosphorylation under these experimental conditions.

These large differences in the promotional tendencies cannot be due to similar differences in the formation degrees of the complexes, because the divalent metal ions of Table I exist in $10^{-3}$ M reactant solutions already at pH 5.5 to at least 50% in the form of $M(ATP)^{2-}$ (cf. ref 31–33); obviously at least the same formation degree must be surmised[5] for the trivalent metal ions. Although the water substitution rates for the divalent aqua metal ions as well as of $Y^{3+}$ and $La^{3+}$ differ considerably,[34] they again cannot be the reason for the different accelerations. This conclusion is also born out from the results obtained for mixed metal ion systems:[4] $Cu^{2+}$/ATP with $Mg^{2+}$, $Ni^{2+}$, or $Cd^{2+}$ reach at pH 5.5 the same limiting rate. Moreover, $(tn)_2Co^{III}$ (if present in excess) is an excellent promoter of the dephosphorylation, and its water-substitution rate is slow compared with the mentioned divalent metal ions and $Y^{3+}$ or $La^{3+}$. It should be pointed out, however, that compared to many related $Co^{III}$ complexes, $(tn)_2Co^{III}$ undergoes water substitution, trans/cis isomerization, and complexation rapidly, especially in the vicinity of pH 6.5 where $(tn)_2Co(OH)(OH_2)^{2+}$ is maximized. Thus, Stranks et al.[28,35] observed that trans → cis isomerization proceeds for the hydroxoaqua complex with a half-life of about 1 s at 25 °C.

What is then the reason that only certain metal ions produce large accelerations in NTP dephosphorylations? For $M^{2+}$/NTP systems, it has been concluded[4] that the most reactive pathway in the dephosphorylation involves an *intra*molecular nucleophilic $OH^-$ attack via a $M-OH^+$ unit already coordinated to the nucleotide (section 1). Accordingly, in ATP systems, the pH at which the maximum promotion occurs increases, beginning with $Cu^{2+}$ at pH 6.7, within the series $Cu^{2+} < Zn^{2+} < Ni^{2+} < Cd^{2+} < Mn^{2+}$ ($<Mg^{2+}$);[4] the tendency of these ions to form hydroxo complexes follows the reverse order.[33,36] The results obtained now for $(tn)_2Co^{III}$ (see also ref 5), $Y^{3+}$, and $La^{3+}$ at pH 5.5 and 7.5 fit into this picture: $(tn)_2Co(OH)(OH_2)^{2+}$ is developed at pH 6.5,[28,35] while for lanthanide ions hydroxo complex formation only begins to occur at this pH.[37] NTP coordination will somewhat inhibit this hydroxo-complex formation, but for the second and unsaturated metal center (see also section 1), the formation of a M–OH unit will still occur at about the same pH. Hence, one of the reasons why different metal ions accelerate the dephosphorylation of NTPs differently relates to their different ability to form hydroxo complexes. A further aspect is their different affinity toward N-7 of ATP (see sections 1 and 4).[4]

**Table II.** Comparison of the Initial Rate of Dephosphorylation, $v_o$ (M $s^{-1}$), of Nucleoside 5′-Triphosphates (NTP) in $10^{-2}$ M Solutions in the Presence of Different Metal Ions ($I$ = 0.1, $NaClO_4$; 50 °C). All Initial Rates Are Given as $v_o \times 10^8$

| | | [$M^{n+}$]/[NTP] (1:1) | | [$M^{n+}$]/[NTP] (2:1) | |
|---|---|---|---|---|---|
| NTP | $M^{n+}$ | pH 5.5 | pH 7.5 | pH 5.5 | pH 7.5 |
| ATP | none | 1.1 | 0.32 | 1.1 | 0.32 |
| | $Ni^{2+}$ | | 5[a] | | 14[a] |
| | $Cu^{2+}$ | 250[a] | 750[a] | 3000[a] | 750[a] |
| | $Zn^{2+}$ | | 25[a] | | 120[a] |
| | $(tn)_2Co^{III}$ | 8.5 | 6.4 | ~5000 | ~4000 |
| UTP | none | 1.1 | 0.32 | 1.1 | 0.32 |
| | $Ni^{2+}$ | | 0.5 | | b |
| | $Cu^{2+}$ | 1.7[c] | 5.6[c] | 7[c] | b |
| | $Zn^{2+}$ | | ~1[d] | | b |
| | $(tn)_2Co^{III}$ | 8.5 | 6.4 | ~5000 | ~3000 |

[a] Extrapolated from the data given in ref 16. [b] Precipitation. [c] Extrapolated from the data given in ref 3; the same results hold also for $Cu^{2+}$/CTP (ref 16). [d] The solution appeared slightly "milky".

**4. Influence of Total Reactant Concentration on the Dephosphorylation Rate.** To see how the total concentration of the reactants influences comparisons between the dephosphorylation rates, we have increased the NTP concentration to $10^{-2}$ M for

(31) Sigel, H. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1903–1911.
(32) Fukuda, Y.; Mitchell, P. R.; Sigel, H. *Helv. Chim. Acta* **1978**, *61*, 638–647.
(33) Sigel, H.; Scheller, K. H.; Milburn, R. M. *Inorg. Chem.* **1984**, *23*, 1933–1938.
(34) (a) Eigen, M. *Pure Appl. Chem.* **1963**, *6*, 97–115. (b) Frey, C. M.; Stuehr, J. E. *Met. Ions Biol. Syst.* **1974**, *1*, 51–116. (c) Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry", 4th ed.; Wiley: New York, 1980; p 1188.
(35) (a) Jonasson, I. R.; Murray, R. S.; Stranks, D. R.; Yandell, J. K. *Proc. Int. Conf. Coord. Chem. 12th* **1969**, 32. (b) Fowless, A. D.; Stranks, D. R. *Inorg. Chem.* **1977**, *16*, 1276–1281.
(36) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209–3214.
(37) Martin, R. B. *Met. Ions Biol. Syst.* **1984**, *17*, 1–49 (see p 33).

some of the systems. The initial rates ($v_0 \times 10^8$ M $s^{-1}$) are listed in Table II for $M^{n+}$/NTP ratios of 1:1 and 2:1 at pH 5.5 and 7.5. For $Cu^{2+}$ and $(tn)_2Co^{III}$ the accelerations are now even more dramatic; e.g., at a 2:1 ratio and pH 5.5, one observes about a 3000-fold increase for $Cu^{2+}$/ATP and roughly a 5000-fold increase for $(tn)_2Co^{III}$/ATP or UTP compared with the nonpromoted reaction. However, it is also evident that the promotion of the dephosphorylation rate in the $Cu^{2+}$/UTP system is considerably less pronounced: the increase is only about 7-fold.

In previous studies on $M^{2+}$/NTP systems, the dependence of the dephosphorylation rates on the concentrations of the reactants was used to establish the nature of the reactive species (section 1; see Figure 7 in ref 4). For $Cu^{2+}$/UTP or CTP systems, it is a complex of the type $Cu_2(NTP)(OH)^-$, where the nucleic base moiety of the pyrimidine-NTP is not involved; consequently, the $Cu^{2+}$/methyl triphosphate system behaves alike.[3,4,16] For $Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$, or $Cd^{2+}$/ATP systems,[4,16] the reaction proceeds by way of dimeric complexes such as $[M_2(ATP)]_2(OH)^-$;[4] the formation of the dimers occurs via base-stacking[38] and depends on the interaction between the metal ions and N-7 of the base moiety of ATP.[4] Hence, for a 2:1 $M^{2+}$/NTP ratio, equilibria 1 and 2 are of importance for all systems, while equilibrium 3 is of importance only for ATP (and partly also other purine-NTP) systems. In other words, the rate-determining step of the most efficient dephosphorylation process occurs for $M^{2+}$/pyrimidine-NTP systems, including all other triphosphate esters with only a metal ion–phosphate interaction, after equilibrium 2 and hydroxo-complex formation, while for the indicated $M^{2+}$/ATP systems this step occurs only after equilibrium 3 and hydroxo-complex formation (for details see ref 4). Consequently, the initial rate for pyrimidine-NTPs[3,16] is proportional to the concentration of $M^{2+}$/NTP (i.e., a simple first-order dependency is obtained), while for ATP[4,16] it is proportional to the square of the concentration of $M^{2+}$/ATP (i.e., a second-order dependency is observed).

$$M^{2+} + NTP^{4-} \rightleftarrows M(NTP)^{2-} \quad (1)$$

$$M^{2+} + M(NTP)^{2-} \rightleftarrows M_2(NTP) \quad (2)$$

$$2M_2(NTP) \rightleftarrows [M_2(NTP)]_2 \quad (3)$$

Tables I and II reflect these reaction patterns. Thus, for the $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$/UTP systems, we observe a 10-fold rate increase; e.g., for $Cu^{2+}$ at the 2:1 ratio and pH 5.5, the initial rate $v_0$ goes from $0.7 \times 10^{-8}$ M $s^{-1}$ (Table I) to $7 \times 10^{-8}$ M $s^{-1}$ (Table II). Furthermore, for the $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$/ATP systems, a 100-fold increase occurs; e.g., for $Cu^{2+}$ at the 2:1 ratio and pH 5.5, $v_0$ goes from $30 \times 10^{-8}$ M $s^{-1}$ (Table I) to $3000 \times 10^{-8}$ M $s^{-1}$ (Table II).

With $(tn)_2Co^{III}$, the matter is more complicated (Tables I and II): although the ATP and UTP systems behave within experimental error alike, the initial rate increases clearly more than 10-fold, but less than 100-fold, in going from NTP concentrations of $10^{-3}$ to $10^{-2}$ M. In the 1:1 systems, the increase is about 20-fold at pH 5.5 and 40-fold at pH 7.5, while in the 2:1 systems it is about 40- and 50-fold at the respective pH values. As a first approximation one may conclude that for 2:1 systems in the NTP-concentration range of $10^{-3}$ to $10^{-2}$ M, the proportionality between initial rate and reactant concentration is represented by $v_0 \propto [2(tn)_2Co^{III}/NTP]^{1.6}$. This value will be used in section 8.

**5. Some Considerations on the Reactive Species in $(tn)_2Co^{III}$/NTP Systems.** The 40- to 50-fold rate increase for the $(tn)_2Co^{III}$/NTP 2:1 systems on going from $10^{-3}$ to $10^{-2}$ M NTP concentrations could be accomodated by a preequilibrium scheme of the type shown by reactions 1–3 involving monomeric and dimeric reactive species (section 4). However, dimer formation of the type important for $M^{2+}$/ATP systems cannot be suggested for $(tn)_2Co^{III}$ because the rate behavior of the ATP and UTP systems is now identical within experimental error (Tables I and II), while the stacking capabilities of adenine and uridine moieties are very different.[38,39] In addition, for $(tn)_2Co^{III}$/ATP, it is difficult to see how a $Co^{III}$/N-7 interaction would be possible in a dimer of the type $[M_2(ATP)]_2$ (see Figure 7 in ref 4), because the $(tn)_2Co^{III}$ unit provides only two accessible coordination positions, and these are expected to be occupied by the phosphate chain.[5]

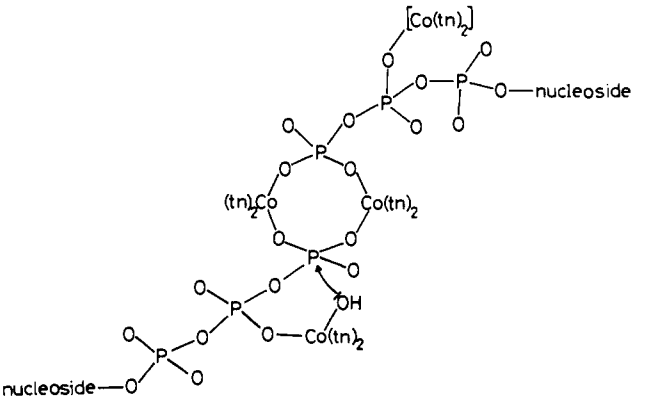



**Figure 2.** Possible structure for a dimeric $[(tn)_2Co]_4(NTP)_2(OH)^{3+}$ species involving an eight-membered ring (see text in section 5).

However, one could suggest a dimer related to the well-characterized $[(en)_2Co(\mu\text{-}O_3POC_6H_5)]_2^{2+}$ which involves an eight-membered ring.[40] Figure 2 shows such a possibility; this complex could allow for $PO_4$ production through intramolecular attack of a $\gamma$ phosphorus by coordinated hydroxide (one of the four cobalts, not required for dephosphorylation, is shown in parenthesis). The indicated eight-membered ring structure involving dimer formation appears to be rather common in the solid state where it has been found for several ternary complexes, e.g., those containing $Cu^{2+}$ and, as a second ligand, a nucleoside monophosphate[41–43] or ATP.[44] However, it seems to us that the situation with these phosphate complexes[45] may well parallel that for acetate and other carboxylate complexes: many carboxylate-bridged dimers can be prepared in the solid state,[46–48] but no hint exists for the development of the corresponding dimers in aqueous solution.[48–50]

Another possibility to explain the observed concentration dependence is the development of a reactive 3:1 $(tn)_2Co^{III}$/NTP complex. For the $(tn)_2Co^{III}$/pyrophosphate system, direct indication for a 3:1 complex has been obtained from $^{31}P$ NMR studies.[24] In addition, kinetic results for the pyrophoshate and the $(tn)_2Co^{III}$/ATP systems[5] have been interpreted on the basis of 3:1 as well as 2:1 complexes. These earlier studies were conducted at higher concentrations ([ATP] = (1.0–8.6) $\times 10^{-2}$ M

(38) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260.
(39) Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 5891–5900.
(40) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M.; Snow, M. R. *Inorg. Chem.* **1982**, *21*, 4155–4160.
(41) Fischer, B. E.; Bau, R. *Inorg. Chem.* **1978**, *17*, 27–34.
(42) Aoki, K. *J. Am. Chem. Soc.* **1978**, *100*, 7106–7108.
(43) Wei, C.-Y.; Fischer, B. E.; Bau, R. *J. Chem. Soc., Chem. Commun.* **1978**, 1053–1055.
(44) Sheldrick, W. S. *Angew. Chem.* **1981**, *93*, 473–474; *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 460.
(45) (a) We are not aware of any evidence for the development of these eight-membered rings in aqueous solution, although with Co(III) when such ring systems are preformed (e.g., in another solvent) they can have a long-term existence in water. In the case of $Cu^{2+}$ and other divalent metal ions and several nucleoside monophosphates, no indications for such dimers have been observed;[45b] despite variation of the total concentrations, the experimental data of the potentiometric pH titrations can be fitted perfectly with monomeric species only. Similarly, for $M^{2+}$/NTP systems, no hint exists for the formation of dimers of this type[31,33] (although association via the base moieties is known[38,39]). (b) Sigel, H.; Scheller, K. H. *Eur. J. Biochem.* **1984**, *138*, 291–299.
(46) Doedens, R. J. *Prog. Inorg. Chem.* **1976**, *21*, 209–231.
(47) See ref 34c, pp 817–818.
(48) Dubler, E.; Häring, U. K.; Scheller, K. H.; Baltzer, P.; Sigel, H. *Inorg. Chem.* **1984**, *23*, 3785–3792.
(49) Sillén, L. G.; Martell, A. E. "Stability Constants of Metal-Ion Complexes"; The Chemical Society: London, (a) 1964, Spec. Publ. No. 17; (b) 1971, Spec. Publ. No. 25.
(50) (a) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York and London, 1977; Vol. 3. (b) Perrin, D. D. "Stability Constants of Metal-Ion Complexes. Part B: Organic Ligands"; Pergamon Press: Oxford, 1979.

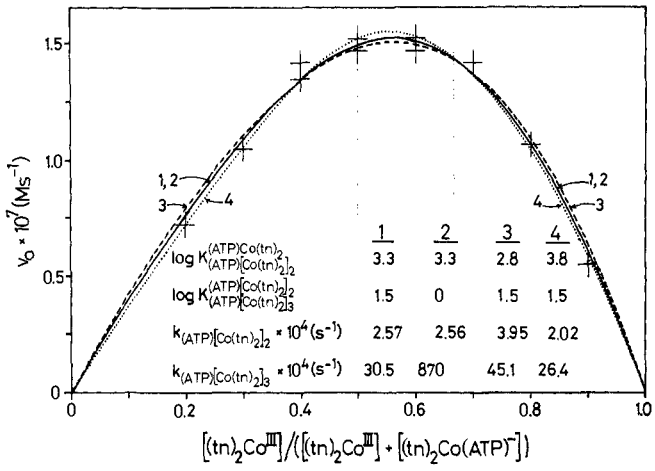



**Figure 3.** Job's series for the $(tn)_2Co^{III}/(tn)_2Co(ATP)^-$ system (with preformed $(tn)_2Co(ATP)^-$; see Experimental Section) at $pH_0$ 6.50. $[(tn)_2Co^{III}]_{tot} + [(tn)_2Co(ATP)^-]_{tot}$ = constant = $2 \times 10^{-3}$ M; $I$ = 0.1, $NaClO_4$, 25 °C. The two vertical dotted lines give the positions of the ratios $(tn)_2Co^{III}/(tn)_2Co(ATP)^-$ = 1:1 or 2:1 (see text in section 5). The crosses (with calculated error bars) represent the experimental data which were analyzed by a curve-fitting procedure, with a Newton–Gauss nonlinear least-squares method, on a Hewlett-Packard 9825A calculator connected to a 7470A calculator–plotter and a 82905B printer. Curve 1 (full) was calculated with the stability constants estimated earlier[5] (see the table inserted into the figure). Curve 2 (practically identical with curve 1; full) shows the influence of the species $[(tn)_2Co]_3(ATP)^{5+}$ on the results by reducing its stability constant from 30 $M^{-1}$ (=1.5 log units) to 1 $M^{-1}$ (=0 log units) and the existing coupling with the corresponding rate constant $k_{(ATP)[Co(tn)_2]_3}$. Curves 3 (broken) and 4 (dotted) indicate the effect of a possible error in the stability constant of $[(tn)_2Co]_2(ATP)^{2+}$ on the results (the error limits of ±0.5 log units are based on previous experience[16]). These calculations demonstrate that a satisfactory fit of the experimental data is obtained in all four cases, i.e., as long as a reactivity is attributed to $[(tn)_2Co]_2(ATP)^{2+}$ *and* $[(tn)_2Co]_3(ATP)^{5+}$; they demonstrate further that a fit without the species $[(tn)_2Co]_3(ATP)^{5+}$ is not possible because then the *calculated* maximum of the rate is obtained at a reactant ratio of 1:1, which is in fact clearly not observed.

and $[(tn)_2Co^{III}]$ = (1–30) × $10^{-2}$ M), i.e., under conditions more favorable to higher degrees of complex formation. In the present study, a 3:1 complex would be more favored for the $10^{-2}$ M (Table II) than for the $10^{-3}$ M NTP solutions (Table I).

To learn more about the possible involvement of a reactive 3:1 complex in the $(tn)_2Co^{III}$/NTP system, we have carried out a series of measurements according to Job's method[51] at pH 6.5 and 25 °C by using the preformed $(tn)_2Co(ATP)^-$ complex. The initial rates, $v_0$ (M $s^{-1}$), were measured and plotted vs. the ratios $[(tn)_2Co^{III}]/([(tn)_2Co^{III}] + [(tn)_2Co(ATP)^-])$, keeping $[(tn)_2Co^{III}] + [(tn)_2Co(ATP)^-]$ constant (=$2 \times 10^{-3}$ M). A maximum rate at the values 0.5 or 0.67 would indicate a composition for the reactive species of $(tn)_2Co^{III}/(tn)_2Co(ATP)^-$ = 1:1 or 2:1, respectively; this means that the reactive species would have a $(tn)_2Co^{III}$/ATP ratio of 2:1 or 3:1. The experimental results and the calculations presented in Figure 3 show that the data can only be explained by attributing reactivities to *two* complexes, which have the compositions $[(tn)_2Co]_2(ATP)^{2+}$ and $[(tn)_2Co]_3(ATP)^{5+}$ (of course, at least part of the positive charges in these two species at pH 6.5 would be compensated by coordinated $OH^-$). Hence it appears, in accord with the previous study,[5] that a 3:1 $(tn)_2Co^{III}$/ATP complex indeed plays a role in the $(tn)_2Co^{III}$/ATP system. However, it is also evident that for final conclusions, more experimental information is needed.[52]

**6. Further Comparisons: The Effect of the Charge of the Metal Center on the Dephosphorylation Rates.** Whatever the factors determining and limiting the reaction rate in the $(tn)_2Co^{III}$/NTP systems may finally be (see section 5), the 2:1 ratio gives a system with a high reactivity toward dephosphorylation. Hence, the question arises what are the reasons for this high reactivity?

As we have seen, the promotion of the dephosphorylation of purine-NTPs, especially of ATP, by $Cu^{2+}$ and other metal ions with a $M^{2+}$/N-7 interaction is much larger than that of pyrimidine-NTPs or of simple triphosphate esters (section 4). This is due to the reduced or even completely missing stacking tendencies of the last mentioned triphosphates and the absence of N-7, or a corresponding binding site, which facilitates the creation of the reactive species described in ref 4.

Such an explanation is clearly not feasible for the $(tn)_2Co^{III}$/NTP systems because here ATP and UTP behave alike. Hence, as in $(tn)_2Co^{III}$/NTP a metal ion/base interaction plays no role (see also section 5), the result should only be compared with the $M^{2+}$/UTP systems if effects other than a $M^{n+}$/base interaction are to be evaluated. Furthermore, because the tendency to form hydroxo complexes, an important feature in dephosphorylations (section 3), is of a similar order for $Cu^{2+}$ and $(tn)_2Co^{III}$, an appropriate comparison is between these two metal ions. Clearly, their charge is obviously different, and the results for the corresponding UTP systems in Table I at pH 5.5 and the 2:1 ratio indicate that this effect is quite significant:[53] $(tn)_2Co^{III}$ is more effective than $Cu^{2+}$ by a factor of about 170.

That the described difference in reactivity is indeed associated with a charge effect is supported by the experiments with $Y^{3+}$ and $La^{3+}$, despite the fact that they were somewhat hampered by precipitation. In the 2:1 ratio and at pH 7.5, all three trivalent metal centers, i.e., including $(tn)_2Co^{III}$, show the same reactivity (Table I). At pH 5.5 in the 2:1 ratio, $Y^{3+}$ and $La^{3+}$ are less effective than $(tn)_2Co^{III}$ by a factor of about 15 due to their lower tendency to form hydroxo complexes. The species $(tn)_2Co(OH)(OH_2)^{2+}$ reaches its maximum formation degree at pH 6.5,[28,35] while with lanthanide ions hydroxo-complex formation only begins at pH >6.5.[37]

**7. Dephosphorylation Process in $M^{n+}$/NTP 1:1 Systems.** Earlier studies[4,16] of 1:1 and 2:1 $M^{2+}$/ATP systems from pH 2 to 10 have shown that the 2:1 mixtures are, with a single exception, always more reactive. The exception is $Cu^{2+}$ at pH ≥6.5: the reactivity of the 1:1 and 2:1 system being equal and the second $Cu^{2+}$ playing no role under these conditions. Table I and II corroborate these results.

The compositions of the several reactive species for $M^{2+}$/NTP systems have previously been determined, including $M_2(RTP)(OH)^-$, $[M_2(ATP)](OH)^-$, and $[Cu(ATP)]_2(OH)^{5-}$ (section 1), and their probable structures have been elucidated.[3,4] The reactivities in the 1:1 systems (with the exception of $Cu^{2+}$/ATP)[4] may be explained by considering the following equilibria:

$$2M(NTP)(OH)^{3-} \rightleftarrows M_2(NTP)(OH)^- + NTP^{4-} + OH^- \quad (4)$$

$$M(NTP)(OH)^{3-} + M(NTP)^{2-} \rightleftarrows M_2(NTP)(OH)^- + NTP^{4-} \quad (5)$$

Assuming that the positions of equilibria 4 and 5 or of any related equilibria are such that a few percent of the dinuclear species (or of their dimers where needed; see section 4 and ref 4) is formed, the reactivity in the 1:1 systems can be fully explained.

The same explanation can be offered for the trivalent metal centers. In $M^{n+}$/NTP 1:1 complexes, the metal ion is certainly coordinated to the $(\alpha),\beta,\gamma$ positions of the triphosphate chain,[54] and the formation of the highly reactive binuclear species is best explained,[3,4] by the coordination of one metal ion to the terminal $\gamma$ phosphate group, with the other metal ion being shifted into the $\alpha,\beta$ position. Such a rearrangement could be somewhat sluggish in $(tn)_2Co^{III}$/NTP systems (though possibly facilitated by excess $M^{n+}$); this provides a possible reason why the corresponding 1:1 systems are so unreactive (Table I). Substitution rates for lanthanides are high,[34] and in accord with the above concept, the dephosphorylation rates in 1:1 systems with $Y^{3+}$ and

(51) Job, P. *C. R. Hebd. Seances Acad. Sci.* **1933**, *196*, 181–183.

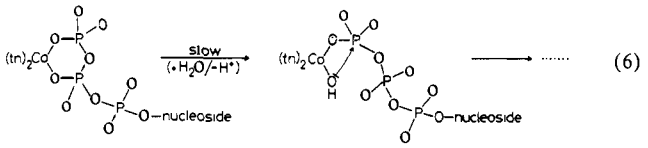
(52) For example, in Job's series with a total reactant concentration of $10^{-4}$ M, the 2:1 species should become more important, while at $10^{-2}$ concentrations the 3:1 species should be dominating.

(53) It should be noted that the larger saturation degree of the coordination sphere of $Co^{3+}$ in $(tn)_2Co^{III}$, compared with the one of $Cu^{2+}$, may even partly diminish the effect.

(54) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57–124.

$La^{3+}$ are quite large compared with the $M^{n+}$-free systems: at pH 7.5, the reactions are facilitated with $Y^{3+}$ or $La^{3+}$ by factors of about 700, while with $(tn)_2Co^{III}$ the factor is only 5.

For the $(tn)_2Co^{III}$/NTP systems, another possibility is that the reaction, in the 1:1 ratio, proceeds from the 1:1 monomeric six-membered $\beta,\gamma$-chelate by way of a relatively slow ring-opening to provide a coordinated hydroxide as indicated in the process 6:




(6)

For the related complex, *cis*-$[(en)_2Co(OH)O_3POC_6H_4NO_2]$, dephosphorylation by means of intramolecular attack on the phosphorus by the coordinated hydroxide has been demonstrated.[26c,55]

**8. "Pitfalls" in Comparing Rates for Different Systems.** The results assembled in Tables I and II can of course be compared directly. One should be aware, however, that any comparison is strictly valid only for those conditions under which the data have been obtained. Any extrapolation, e.g., to other pH regions, is dangerous.

For example, the effectiveness of $Zn^{2+}$ and $Cd^{2+}$ at the $M^{2+}$/ATP 1:1 ratio in $10^{-3}$ M reactant solutions is identical at pH 5.5 and 7.5 (Table I). However, the maximum rates are observed at different pH values: the $Zn^{2+}$ system reaches its peak at pH about 8 (cf. ref 16) and the $Cd^{2+}$ system only at pH 10;[4] i.e., at pH >8, the reactivity in the $Zn^{2+}$/ATP 1:1 system decreases while it is still increasing in the $Cd^{2+}$ system. The consequence is a rather similar promotion of the ATP dephosphorylation by $Zn^{2+}$ and $Cd^{2+}$ in the pH range below 8, while at pH 10 the $Cd^{2+}$ system is more reactive[4] by at least a factor of 50.

Similarly, if the $Cd^{2+}$ and $Cu^{2+}$/ATP 1:1 systems[4] are compared, the promotion by $Cd^{2+}$ at pH 10 is only about $^1/_6$ less pronounced than that by $Cu^{2+}$ at pH 6.5 (the pH where $Cu^{2+}$/ATP reaches its maximal reactivity),[16] while at pH 7.5 $Cd^{2+}$ is less efficient by a factor of $^1/_{30}$ than $Cu^{2+}$ at the same pH (Table I). Moreover, a comparison[4] of the rates at pH 10 indicates a 10-fold *higher* reactivity for the $Cd^{2+}$ system.

Another and more serious problem in attempting extrapolations relates to the *different* proportionalities between the initial rates of the dephosphorylation and the concentrations of the reactants. These different proportionalities have often been neglected in the literature, and therefore false comparisons have been made.

To make this important point more clear, we can use the proportionalities indicated in section 4 (cf. ref 56) to calculate the initial rates at pH 5.5 for several $M^{n+}$/NTP 2:1 systems. The calculated dephosphorylation rate $v_0$ in a $M^{n+}$-free $10^{-1}$ M NTP solution is $1.1 \times 10^{-7}$ M $s^{-1}$, while one calculates $v_0 = 7 \times 10^{-7}$, $3 \times 10^{-3}$, and $2 \times 10^{-3}$ M $s^{-1}$ for the 2:1 systems of $Cu^{2+}$/UTP, $Cu^{2+}$/ATP, and $(tn)_2Co^{III}$/ATP, respectively. There are two points to be noted: (i) Under these conditions ([NTP] = $10^{-1}$ M), $Cu^{2+}$ accelerates the ATP dephosphorylation even more than $(tn)_2Co^{III}$. (ii) The promotion factors for the dephosphorylation in these 2:1 systems (compared with the $M^{n+}$-free systems) are 6.4, $2.7 \times 10^4$, and $1.8 \times 10^4$, respectively. For $10^{-3}$ M NTP solutions, they are 6.4, 270, and 1200 (as calculated from data given in Table I). Hence, for the $Cu^{2+}$/UTP 2:1 system, one arrives at the same promotion factor for the two concentrations, because it is a first-order reaction, while for the 2:1 systems of $Cu^{2+}$/ATP and $(tn)_2Co^{III}$/ATP quite different factors are obtained, because their reaction orders are higher.

It is evident that depending on the reaction conditions one has used, one can arrive at different conclusions: thus at low reactant concentrations, small or moderate accelerations are seen, while at high concentrations large rate enhancements may be "celebrated". Clearly the very worst type of comparison is between different systems, which have been studied at different concentrations, without taking into account the possibly different proportionalities between rate and reactant concentrations.

**Table III.** Promotion of the Initial Rate of Dephosphorylation, $v_0$ (M $s^{-1}$), of the $(tn)_2Co^{III}$/ATP 1:1 System ($10^{-3}$ M) by the Addition of 1 or 5 Extra equiv of $M^{n+}$ at pH 5.5 ($I$ = 0.1, $NaClO_4$; 50 °C). All Initial Rates Are Given as $v_0 \times 10^8$

| extra $M^{n+}$ | $v_0$ at $[M^{n+}]$ = $10^{-3}$ M | $5 \times 10^{-3}$ M |
|---|---|---|
| none | 0.45[a] | 0.45[a] |
| $Mg^{2+}$ | 0.66 | 0.96 |
| $Ni^{2+}$ | 0.40 | 0.81 |
| $Zn^{2+}$ | 1.6 | 3.5 |
| $Cu^{2+}$ | 5.3 | 16 |
| $Cu(bpy)^{2+}$ | 6.9 | 18 |
| $(tn)_2Co^{III}$ | 130[a] | |

[a] Values from Table I.

**9. Dephosphorylation Rates in Systems Containing Two Different Metal Ions.** From the results, it is evident that the most reactive species contain two or more metal ions per NTP (sections 2 and 5). To see if the addition of a metal ion different from the one already present in a $M^{n+}$/$ATP^{4-}$ 1:1 complex influences the dephosphorylation rate, we studied at pH 5.5 the effect of various divalent metal ions on the dephosphorylation of the $(tn)_2Co^{III}$/ATP system. We selected this system because the addition of one further equivalent of $(tn)_2Co^{III}$ to the $(tn)_2Co(ATP)^-$ complex promotes the dephosphorylation rate rather dramatically, and it seemed interesting to learn something about the effect of other ions.

It is evident from Table III that the addition of $Mg^{2+}$ and $Ni^{2+}$ to $(tn)_2Co(ATP)^-$ has nearly no effect on the dephosphorylation rate. With $Zn^{2+}$, the situation is somewhat better: if a 5-fold excess is used, a nearly 8-fold acceleration is achieved. A moderate promotion by a factor of about 40 results with a 5-fold excess of $Cu^{2+}$ and $Cu(bpy)^{2+}$. However, compared with the nearly 300-fold rate enhancement of a single equivalent of $(tn)_2Co^{III}$, this is not impressive.

The latter result indicates again that the charge of the metal center is important (section 6): most probably the extent of complex formation is greater for a 1-/3+ interaction than for a 1-/2+ interaction. In line with this interpretation, $Cu(bpy)^{2+}$ is observed to be somewhat more effective than $Cu^{2+}$ in promoting the dephosphorylation of the $(tn)_2Co(ATP)^-$ complex. This result reflects the well-known increased coordination tendency of $Cu(bpy)^{2+}$, compared with $Cu^{2+}_{aq}$, toward the phosphate moiety and other ligands offering O as donor atoms.[57,58] That $Cu(bpy)^{2+}$ can be a better promoter for certain reactions than $Cu^{2+}$ has also been observed before.[57]

The results obtained with $Cu(bpy)^{2+}$ are also fascinating from a mechanistic point of view. $Cu(bpy)^{2+}$ has only two strongly coordinating equatorial binding sites left; from its influence on

(55) However, one has to mention also that for the 1:1 ratio of $(tn)_2Co^{III}$/NTP an increase in concentration from $10^{-3}$ M (Table I) to $10^{-2}$ M (Table II) results in $v_0$ increases of 20-fold (pH 5.5) and 40-fold (pH 7.5) and *not* in the 10-fold increase expected if either the "ring opened 1:1 complex" (eq 6) or the "2:1 dinuclear complex" (eq 4 and 5) provide the *sole* dephosphorylation path. The observed dependence on concentration would be consistent with a combination of the two suggestions, i.e., the simultaneous importance of a 1:1 and 2:1 $(tn)_2Co^{III}$/ATP complex, if the 2:1 complex would become more important at higher concentrations. A further possibility would be the additional presence of a reactive 3:1 $(tn)_2Co^{III}$/ATP complex (see section 5) or a reactive 2:2 dimer; such species could also become more important at the higher concentration level. Additional experiments will be needed to test and evaluate the several possibilities.

(56) This means that we are assuming that the proportionalities given in section 4 are not only valid in the NTP concentration range $10^{-3}$ to $10^{-2}$ M, but also in the range of $10^{-2}$ to $10^{-1}$ M. This assumption is certainly correct for $v_0 \propto$ [NTP] and $v_0 \propto [Cu^{2+}/UTP]$, but for $v_0 \propto [Cu^{2+}/ATP]^2$ and $v_0 \propto [(tn)_2Co^{III}/ATP]^{1.6}$, the proportionalities might deviate somewhat toward linearity (i.e., first-order reactions) due to the beginning of "saturation"; this problem has so far not been studied for these systems.

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the reaction rate, compared with $Cu^{2+}$, it is suggested that this is enough, but also that both of these binding sites are used for coordination to the triphosphate chain. As the reaction most probably proceeds also in this case via an intramolecular nucleophilic $OH^-$ attack from a M-OH unit (section 3), one may tentatively suggest the following structure and mechanistic path for the reactive $[(tn)_2Co(OH)(ATP)Cu(bpy)]^0$ species: $Cu(bpy)^{2+}$, coordinating to the $\alpha,\beta$ phosphate group of $ATP^{4-}$, leads to ring-opening of the cobalt chelate and to a monodentate coordination of $(tn)_2Co(OH)^{2+}$ at the $\gamma$ group, thus allowing for an intramolecular attack at the $\gamma$ phosphorus by the cobalt-bound $OH^-$.

In conclusion, it is evident that systems containing two different metal ions can also be rather reactive toward the dephosphorylation of nucleoside 5'-triphosphates. This observation corresponds to many biological phosphoryl and nucleotidyl transfers, where two different metal ions are also often involved.[9,10]

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**Registry No.** ATP, 56-65-5; UTP, 63-39-8; $Mg^{2+}$, 22537-22-0; $Mn^{2+}$, 16397-91-4; $Ni^{2+}$, 14701-22-5; $Cu^{2+}$, 15158-11-9; $Cu(bpy)^{2+}$, 16482-45-4; $Zn^{2+}$, 23713-49-7; $Cd^{2+}$, 22537-48-0; $(tn)_2Co^{III}$, 95842-01-6; $Y^{3+}$, 22537-40-2; $La^{3+}$, 16096-89-2.

# Crystal and Solution Structures of *cyclo*(Ala-Pro-Gly-D-Phe-Pro): A New Type of Cyclic Pentapeptide Which Undergoes Cis-Trans Isomerization of the Ala-Pro Bond

**Lila M. Gierasch,*† Isabella L. Karle,*‡ Arlene L. Rockwell,† and Kemal Yenal†**

*Contribution from the Department of Chemistry, University of Delaware, Newark, Delaware 19716, and the Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375.* *Received September 13, 1984*

**Abstract:** *cyclo*(L-Ala-L-Pro-Gly-D-Phe-L-Pro) has been synthesized and its conformation determined in the crystalline state and in solution in chloroform and dimethyl sulfoxide. This cyclic pentapeptide was designed to explore the conformational response of the restricted pentapeptide ring to the presence of an L residue preceding proline; it is the first cyclic pentapeptide with an LLDDL chiral sequence to be studied. In crystals, a cis Ala-Pro bond exists, and there are no intramolecular hydrogen bonds. The Ala NH is buried in the interior of the backbone ring and does not participate in any hydrogen bonding. In both prolines, the $C^\gamma$ atom is disordered between at least two positions corresponding to two different envelope conformations for the pyrrolidine ring. The peptide crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters $a$ = 9.142 (2) Å, $b$ = 11.000 (4) Å, and $c$ = 23.885 (5) Å. In solution in dimethyl sulfoxide, the same one-cis form of the peptide is observed, but a conformational change occurs to an all-trans form in chloroform. There appear to be hydrogen-bonding interactions within the ring in the all-trans form, but they are not well-defined. The most likely conformation based on proton and carbon NMR data for the all-trans form contains a type II Pro-Gly $\beta$ turn within which is a weak $\gamma$ turn with a hydrogen bond between the Gly NH and the Ala C=O.

Cyclic pentapeptides have been extensively studied as conformational models, particularly for reverse turns.[1-8] In cases where both solution and crystal structure analyses have been carried out (Table I), an all-trans conformation containing an internal 4 → 1 hydrogen bond in a type I or II $\beta$ turn and often an internal 3 → 1 hydrogen bond in a $\gamma$ turn is observed for this family of peptides. Yet all these examples fall into a single class of backbone sequence chirality: DLLDL or its mirror image LDLLD (with Gly residues occupying either L or D sites).

The peptide *cyclo*(Ala-Pro-Gly-D-Phe-Pro) (all residues are of the L configuration unless otherwise noted) was originally designed to explore a question raised in the solution conformational analysis of *cyclo*($Gly^1$-$Pro^2$-$Gly^3$-D-$Ala^4$-$Pro^5$).[2] While a predominant all-trans conformer with both $\beta$ and $\gamma$ turn intramolecular hydrogen bonds exists for this latter peptide in several solvents, a small proportion (<20%) of a conformer containing one-cis peptide bond is observed in water. Complexation with cations perturbs the conformational distribution, leading to higher proportion of the one-cis form. Model building led to the proposal that it is the $Gly^1$-$Pro^2$ bond that undergoes isomerization in *cyclo*(Gly-Pro-Gly-D-Ala-Pro). Furthermore, introduction of an L residue in place of $Gly^1$ was predicted to favor the one-cis form; replacement of $Gly^1$ with a D residue was predicted to preclude formation of the one-cis form. These predictions have been substantiated by synthesis of the title peptide and by synthesis and conformational analysis of *cyclo*(D-Phe-Pro-Gly-D-Ala-Pro). No cis form has been seen for this latter peptide, even upon complexation by cations.[11] A complete conformational analysis

†University of Delaware.
‡Naval Research Laboratory.

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