(46) (a) J. H. Burchenal, H. H. Adams, N. S. Newell, and J. J. Fox, *Cancer Res.*, **26**, 370 (1966); (b) H. N. Prince, E. Grunberg, M. Buck, and R. Cleeland, *Proc. Soc. Exp. Biol. Med.*, **130**, 1080 (1969).
(47) A. P. Martinez, W. W. Lee, and L. Goodman, *J. Med. Chem.*, **9**, 268 (1966).
(48) (a) W. Steglich and G. Höfle, *Angew. Chem., Int. Ed. Engl.*, **8**, 981 (1969); (b) M. J. Robins, R. A. Jones, and M. MacCoss, *Biochemistry*, **13**, 553 (1974).
(49) R. J. Cushley, I. Wempen, and J. J. Fox, *J. Am. Chem. Soc.*, **90**, 709 (1968).
(50) A. F. Cook and J. G. Moffatt, *J. Am. Chem. Soc.*, **89**, 2697 (1967).
(51) M. J. Robins, R. Mengel, R. A. Jones, and Y. Fouron, *J. Am. Chem. Soc.*, in press; J. N. Brown and L. M. Trefonas, *Org. Prep. Proced.*, **2**, 317 (1970).
(52) J. Beránek and J. Pitha, *Collect. Czech. Chem. Commun.*, **29**, 625 (1964).

Hydrolysis of Nucleoside Phosphates. 6.[1] On the Mechanism of the Metal Ion Promoted Dephosphorylation of Purine Nucleoside 5′-Triphosphates

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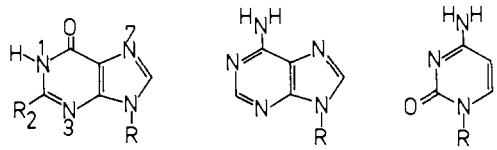
Abstract: The first-order rate constants (50 °C; $I = 0.1$, $NaClO_4$) for the dephosphorylation of ATP in M^{2+}–ATP 2:1 systems ($M^{2+} = Ni^{2+}$, Cu^{2+}, or Zn^{2+}) were compared with those of the 1:1 systems. For all three metal ions the dephosphorylation of ATP is enhanced by a second equivalent of M^{2+}. This has initiated measurements of the initial rate, $v_0 = d[PO_4^{3-}]/dt$, of the ATP hydrolysis as a function of increasing $[M^{2+}]$; these and studies by Job's method have evidenced that in the Cu^{2+}–ATP system at pH 6.7 a 1:1 complex is the most reactive species; however, at pH 4.5 and in systems with Zn^{2+} and Ni^{2+} optimal enhancement of the rate occurs with a M^{2+}–ATP ratio of 2:1. Next, the relation between v_0 and the concentration of M^{2+}–ATP in 1:1 and 2:1 systems was determined, and a square dependence was observed; this with previous results of the ATP, ITP, and GTP (=NTP) systems evidences that the reactive complexes are dimers, i.e., depending on conditions $[M(NTP)]_2^{4-}$ or $[M_2(NTP)]_2$. Their structures are deduced; the purine moieties are stacked and a shift of the M^{2+} along the phosphate backbone of one NTP into an α,β coordination is induced by coordination of this metal ion to N(7) of the other NTP thus leading to a γ-phosphate group ready for nucleophilic attack by OH^- or H_2O; this group is further labilized by coordination of an additional M^{2+}. This mechanism is consistent with results from the CTP system ($v_0 \sim [Cu(CTP)^{2-}]$ or $[Cu_2(CTP)]$), which is of low reactivity (comparable with Cu^{2+}–methyltriphosphate) indicating that the base moiety is not participating in the reaction. Pathways for these dephosphorylations, and also those of hydroxylated complexes like $M(NTP)(OH)^{3-}$, are suggested. The connection between in vitro and in vivo *trans*-phosphorylations is discussed amd mechanisms which may operate in nature are outlined.

The enzyme-catalyzed transfers of phosphoryl and nucleotidyl groups are among the most fundamental processes in biochemistry.[2] As the enzymes catalyzing these reactions require divalent metal ions for activity, it is not surprising that an intensive search for models which allow the evaluation of the influence of these metal ions has been undertaken.[2-4] Among the possible roles a metal ion may play in accelerating these transfers are[2-4,6] (a) charge neutralization or shielding, (b) polarization or electron sink, (c) strain induction, (d) template formation for orienting substrates and enzymic catalytic groups, (e) coordination to the leaving group, and (f) relatively tight coordination to the transition state.

The easiest way to obtain more detailed insight into these reactions is to study the transfer of a phosphoryl group to a water molecule, e.g., the hydrolysis of an organic triphosphate[8] or phosphoryl pyrophosphate,[4,9] and it has long been known that the hydrolysis of ATP[10] to ADP and PO_4^{3-} is promoted by divalent metal ions.[11-17] However, the results on the composition and structure of the reactive M^{2+}–ATP complexes are contradictory and equivocal.[18] Some authors concluded that in the active complex the metal ion should be coordinated to the β- or to the α- and β-phosphate groups[9,12,13] and that an interaction between the purine moiety,[12] i.e., N(7),[13] and the metal ion is required. Others deduced that hydroxide-bridged dimeric species of the kind $[M(ATP)(OH)]_2^{6-}$, M = Cu^{2+} or Zn^{2+}, are reactive[14,15] and that the metal ion is *not* bound to the adenine group in the active complex, the function of the adenine moiety being ring stacking in the dimer to facilitate the transition state.[15,16] It has also been suggested that a complex containing two metal ions per triphosphate may be the reactive species, thus resembling a tetrasubstituted pyrophosphate.[8]

To overcome these difficulties we started a systematic study of the structure of binary and ternary nucleoside 5′-triphosphate complexes[19-22] and of their tendencies for dephosphorylation; the Cu^{2+}-promoted dephosphorylation of ADP or ATP[23] was compared with that of CDP, GDP, and IDP or CTP, GTP, and ITP;[24] as the metal ion–nucleic base interaction differs in their complexes, the importance of this interaction for an enhanced rate can be studied. As Mn^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} coordinate differently to the triphosphate chain of ATP[25] and have different tendencies to coordinate to the base moieties,[21] the dephosphorylation of nucleotides in

Chart I



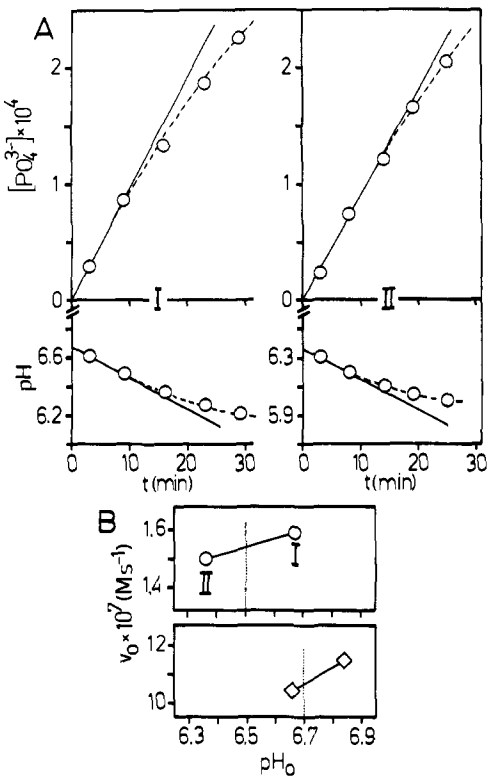


Figure 1. Determination of the initial rate, v_0 (Ms^{-1}), and the corresponding initial pH, pH$_0$ (part A), and the interpolation for v_0 at pH$_0$ 6.5 (part B). A: Cu^{2+}-promoted hydrolysis of ATP; $[ATP]_{tot(t=0)} = [Cu^{2+}]_{tot} = 10^{-3}$ M; $I = 0.1$, $NaClO_4$; 50 °C. Initial rate for case I and extrapolation to $t = 0$ (cf. text), $v_0 = d[PO_4^{3-}]/dt = 1.91 \times 10^{-4}/(20 \times 60) = 1.59 \times 10^{-7}$ Ms^{-1} (pH$_0$ 6.67), and for case II, $v_0 = 1.80 \times 10^{-4}/(20 \times 60) = 1.50 \times 10^{-7}$ Ms^{-1} (pH$_0$ 6.36). B: interpolation from the above results (○) at pH$_0$ 6.50 gives for $v_0 = 1.54 \times 10^{-7}$ Ms^{-1} (cf. Figure 6). An analogous interpolation (◇) for different conditions is additionally given for pH$_0$ 6.70, where $v_0 = 1.065 \times 10^{-6}$ Ms^{-1} (cf. Figure 9).

the presence of these metal ions was also investigated. [1]The dephosphorylation qualities of the purine nucleotides were found to be related, and so we concentrated our attention on ATP, using the other nucleotides for comparison (cf. Chart I). The aim is to evaluate the effect of different concentrations and of different ratios of [NTP]:[M^{2+}] on the rate of dephosphorylation. It is thus possible to give a relatively detailed account of the structure of the reactive metal ion–nucleotide complexes and to relate their properties to those of enzymic systems.

Experimental Section

The materials,[1,23] the apparatus,[23] and the experimental procedures[23] were the same as used recently.

As buffers inhibit the metal ion accelerated dephosphorylation,[1] the pH of the solutions was adjusted with NaOH or $HClO_4$ using a glass stick (the change in volume was negligible). The concentration of free phosphate was determined with molybdate reagent in samples taken at suitable intervals.[23] [NTP] at the time, t, is given by $[NTP]_t = [NTP]_0 - [PO_4^{3-}]_t$, where $[NTP]_0$ is the initial concentration of NTP, and $[NTP]_t$ and $[PO_4^{3-}]_t$ are at the time, t. The free PO_4^{3-} initially present[1] was taken into account in the calculations, whereas the amounts of pyrophosphate and AMP formed are negligible.[12]

The first-order rate constant, k (s^{-1}), was determined from the slope of the straight line portion of a log $[NTP]_t$–time plot. The corresponding pH_{av} was obtained by averaging the pH values measured for those samples that gave points on the straight-line portion. Examples are shown in the figures of ref 1, 23, and 24.

In several cases an exact relationship between rate and pH was essential and to eliminate uncertainties that might arise from the variation of pH or from side reactions, the following procedure was used. The initial rate of dephosphorylation, $v_0 = d[PO_4^{3-}]/dt$ (Ms^{-1}), was determined from the slope of the tangent of the $[PO_4^{3-}]_t$–time curve at the time, $t = 0$. The corresponding initial pH of the reaction

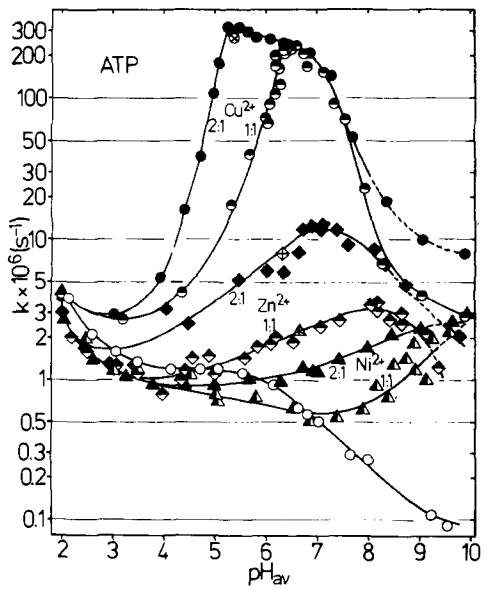


Figure 2. First-order rate constant, k (s^{-1}), for the dephosphorylation of ATP: ATP alone (○),[23] Cu^{2+}–ATP (1:1) (⊖),[23] Cu^{2+}–ATP (2:1) (●) (⊗[13]), Zn^{2+}–ATP (1:1) (♦),[1] Zn^{2+}–ATP (2:1) (◆) (♦[13]), Ni^{2+}–ATP (1:1) (▲),[1] and Ni^{2+}–ATP (2:1) (▲) (▲[13]). $[ATP]_{tot} = 10^{-3}$ M; $I = 0.1$, $NaClO_4$; 50 °C. The dashed lines indicate uncertainty due to precipitation.

solution, i.e., pH$_0$, was determined analogously.[23,24] To obtain v_0 of a given system at a particular pH$_0$, two experiments were carried out in this range of pH, as is shown for a typical pair of measurements in the upper parts of Figure 1; these results were then interpolated to the desired pH$_0$ as shown in the lower part of the same figure.

Results

Dephosphorylation in M^{2+}–ATP 2:1 Systems. Following the suggestion of Miller and Westheimer[8] that "a complex containing two metal ions per triphosphate may be the reactive species" the first-order rate constants, k (s^{-1}), for the dephosphorylation of ATP in the presence of Cu^{2+}, Zn^{2+}, or Ni^{2+}, where $[M^{2+}] = 2[ATP]$, were determined at 50 °C in the pH range 2–10. These results together with those of the corresponding 1:1 systems, which were measured earlier[1,23] and also confirmed now, are plotted in Figure 2.

Figure 3 shows the formation of complexes in 1:1 systems of ATP with Cu^{2+}, Zn^{2+}, or Ni^{2+} in dependence on pH.[26] The Cu^{2+}–ATP 1:1 system has a rate profile with a pH optimum (Figure 2) in the pH range where $Cu(ATP)^{2-}$ strongly dominates, while the decreasing rate of dephosphorylation corresponds to the increasing concentration of $Cu(ATP)(OH)^{3-}$.[23] Though the Zn^{2+}–ATP 1:1 system is less reactive, its general features resemble those of the Cu^{2+} system.[1] However, the Ni^{2+}–ATP 1:1 system shows no pH optimum and the rate increases with increasing $[Ni(ATP)(OH)^{3-}]$. This different behavior has been attributed[1] to the different coordination of these metal ions to the phosphate backbone of ATP.[27] While Cu^{2+} and Zn^{2+} coordinate mainly to the β- and γ-phosphate groups,[25] Ni^{2+} coordinates to all three.[28]

A comparison of the rates of the Cu^{2+}–ATP 2:1 with the 1:1 system (Figure 2) reveals an increased rate for the 2:1 system in the pH range 3.5–6, while at pH $\geq$6.5 the second Cu^{2+} has little or no influence. For Cu^{2+} and for Zn^{2+} the pH optima of the 2:1 systems are about 1 pH unit lower than for the 1:1 systems. However, for Zn^{2+} the reactivity of the 2:1 system is greater than that of the 1:1 over the whole pH range from 3.5 to 10. Although with Ni^{2+} again no pH optimum is observed, the 2:1 system is more reactive from pH 5 to 9.5. We may thus conclude that ATP complexes containing more than one metal ion are indeed more reactive than the 1:1 complexes.

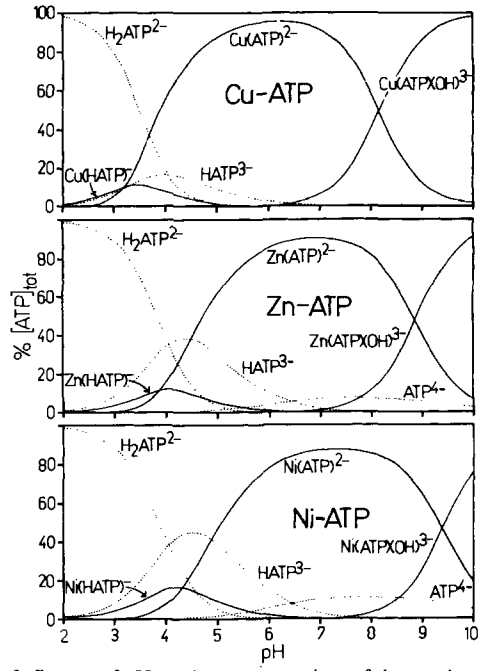


Figure 3. Influence of pH on the concentrations of the species present in an aqueous solution of ATP and Cu^{2+}, Zn^{2+}, or Ni^{2+}, as the percentage of the total ATP or M^{2+} present; computed with the constants listed in ref 1 and 23 (I = 0.1; 25 °C)[26] for concentrations of 10^{-3} M for each reactant. The dotted lines are due to the free ATP species and the solid lines are due to the ATP complexes. Diprotonated complexes of the type $M(H_2ATP)$ were ignored in the calculations as the appropriate constants are unknown. However, such species would probably exist only below pH 3. Upper part: Cu^{2+} and ATP ($[ATP^{4-}] < 1.8\%$). Middle part: Zn^{2+} and ATP. Lower part: Ni^{2+} and ATP.

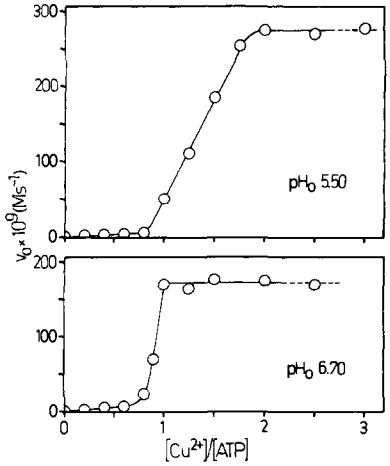


Figure 4. Dependence of the initial rate v_0 of the Cu^{2+}-promoted dephosphorylation of ATP on the ratio $[Cu^{2+}]/[ATP]$. $[ATP]_{tot} = 10^{-3}$ M; I = 0.1, $NaClO_4$; 50 °C. The dashed line portions indicate uncertainty due to precipitation.

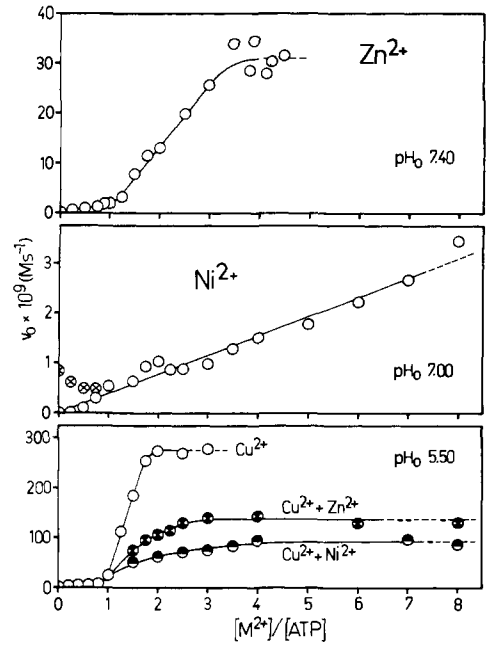


Figure 5. Dependence of the initial rate v_0 on $[M^{2+}]/[ATP]$. $[ATP]_{tot} = 10^{-3}$ M; I = 0.1, $NaClO_4$; 50 °C. The dashed lines indicate uncertainty due to precipitation. Upper part: with Zn^{2+} at pH_0 7.40. Middle part: with Ni^{2+} at pH_0 7.00; in the range $[Ni^{2+}]/[ATP] \leq 0.75$ the measured points (⊗) were corrected for the dephosphorylation of uncomplexed ATP.[29] Lower part: with Cu^{2+} (○) (cf. Figure 4, upper part) and with Cu^{2+} plus Zn^{2+} (●) or Cu^{2+} plus Ni^{2+} (◓) where $[Cu^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M and $[Zn^{2+}]_{tot}$ or $[Ni^{2+}]_{tot} = (0\text{–}7) \times 10^{-3}$ M at pH_0 5.50.

Schneider and Brintzinger[13] have also determined the rate of the three 2:1 systems at a single pH value. These three points are also shown in Figure 2, and although their value for the Ni^{2+} system is about twice our result, the agreement may be considered as excellent.

Dephosphorylation of ATP in Dependence on Increasing Concentrations of M^{2+}. It is now clear that the addition of a second equivalent of M^{2+} to a M^{2+}–ATP 1:1 system enhances the rate. To see if more M^{2+} enhances the rate further and if saturation rates may be reached the following experiments were carried out at constant pH.

At the pH optimum of the Cu^{2+}–ATP 2:1 system at pH_{av} 5.3, the rate is about 15 times faster than the one of the 1:1 system (cf. Figure 2), while at pH_{av} 6.5 the rates are identical. Hence, one expects a rapid increase of the dephosphorylation as $[Cu^{2+}]/[ATP]$ is increased from 1 to 2 at pH 5.3, while at pH 6.5 the rate should remain constant. As these experiments had to be carried out at a constant pH, the only acceptable method was the determination of the initial rate of the dephosphorylation, $v_0 = d[PO_4^{3-}]/dt$ (Ms^{-1}), together with the corresponding pH_0 (cf. Experimental Section). Due to the different evaluation pH_{av} is usually about 0.2 log units smaller than pH_0 (cf. Figure 1 and ref 23 and 24). The results of these experiments (Figure 4, pH_0 5.5 and 6.7) agree well with the predictions and confirm indications from Figure 2 that Cu^{2+}–ATP 2:1 and 1:1 complexes are the reactive species at pH 5.5 and 6.7, respectively.

The rate of dephosphorylation of the Zn^{2+}–ATP 2:1 system dominates the 1:1 system from pH 3.5 through 10 (Figure 2); therefore, the experiments of Figure 5 (upper part) were done at the pH optimum of the 2:1 system, i.e., at pH_0 7.4; a saturation rate is observed at $[Zn^{2+}]/[ATP] \geq 3.5$. With Ni^{2+} the measurements were made at pH_0 7.0 (Figure 5, middle), because at higher pH with even a slight excess of $[Ni^{2+}]$ over [ATP] a precipitate is formed. It is evident that increasing $[Ni^{2+}]$ favors the dephosphorylation of ATP.[29] Though no saturation rate is observed, the existence of Ni_2ATP is known.[27,30]

To investigate further the role of the second metal ion, Zn^{2+} or Ni^{2+} were added in increasing amounts to a Cu^{2+}–ATP 1:1 system at pH_0 5.5. At this pH $Cu(ATP)^{2-}$ is formed to about 90% (cf. Figure 3). As the stability constants are more than ten times smaller for Ni^{2+} and Zn^{2+} than for Cu^{2+},[31] only slight amounts of Cu^{2+} will be displaced from its 1:1 complex under the present conditions, and Ni^{2+} and Zn^{2+} will preferentially coordinate to $Cu(ATP)^{2-}$. The lowest part of Figure 5 shows the remarkable effect of Ni^{2+} and Zn^{2+} on the de-

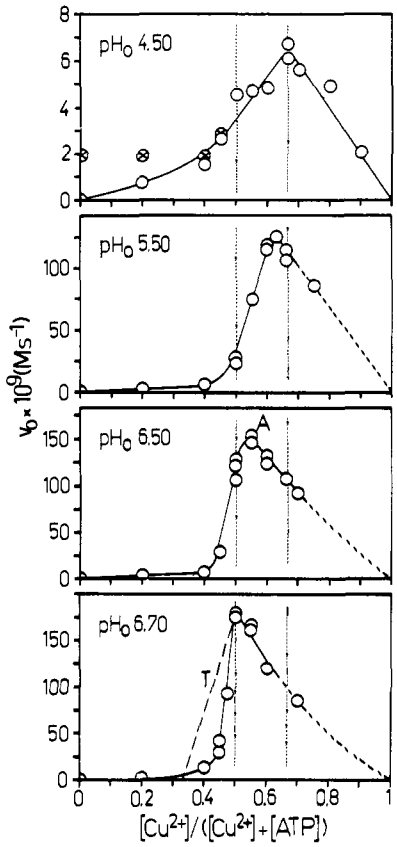


Figure 6. Job's series of the Cu^{2+}-ATP system (cf. text) at different values of pH_0. $[Cu^{2+}]_{tot} + [ATP]_{tot}$ = constant = 2×10^{-3} M; I = 0.1, $NaClO_4$; 50 °C. The dashed line portions indicate uncertainty due to precipitation. The measured points (⊗) were corrected for the rate of dephosphorylation of the present uncomplexed ATP.[29] The point labeled A results from cases I and II in Figure 1. For the meaning of the straight line labeled T in the lowest part of the figure, see text.

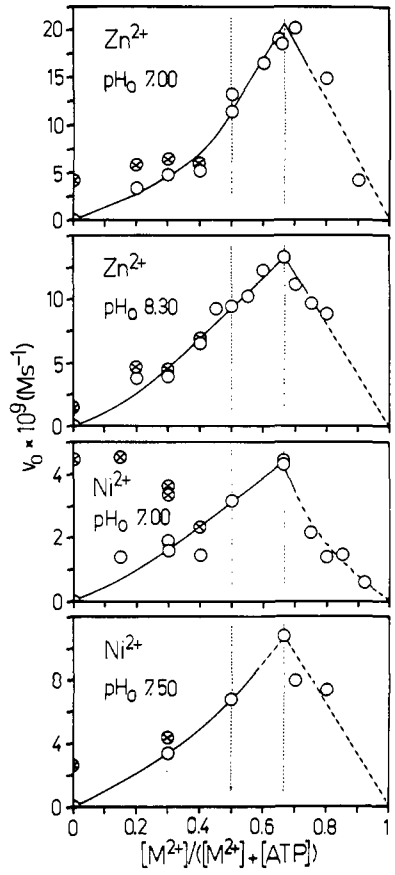


Figure 7. Job's series of Zn^{2+}- and Ni^{2+}-ATP systems. $[M^{2+}]_{tot} + [ATP]_{tot}$ = constant = 5×10^{-3} M; I = 0.1; $NaClO_4$; 50 °C. The dashed lines indicate uncertainty due to precipitation. The measured points (⊗) were corrected for the dephosphorylation of uncomplexed ATP.[29]

phosphorylation rate of the Cu^{2+}-ATP 1:1 system; compared with the Cu^{2+}-ATP 2:1 system the Cu^{2+}/Ni^{2+} or Cu^{2+}/Zn^{2+} systems reach, respectively, a third or a half of the reactivity of the Cu^{2+} 2:1 system. This is remarkable as in the 1:1 systems Ni^{2+} is 300 times, and Zn^{2+} 100 times, less effective in the metal ion promoted dephosphorylation of ATP than is Cu^{2+} (cf. Figure 2),[1] while if used as the second metal ion the additional acceleration of the reaction is nearly as high as with Cu^{2+}.

As it is hard to see how ATP could simultaneously bind more than two metal ions, we assume that the most reactive species has a $[M^{2+}]$:[ATP] ratio of 2:1. This is supported by the data of Figure 4 (upper part), showing a saturation rate at $[Cu^{2+}]$-/[ATP] = 2, as well as by recent demonstrations of the existence of M_2(ATP) for a number of metal ions.[27,30,32,33] For the Ni^{2+} complex holds $\log K^{Ni}_{Ni_2ATP} = 2.4$,[30] while the corresponding Zn^{2+} complex has been observed by NMR.[33] Assuming that Zn_2ATP, or a related species occurring only in low concentrations (e.g., a dimer thereof), is the reactive complex and that complete formation of $Zn(ATP)^{2-}$ under the 1:1 conditions (cf. Figure 3) occurs, one estimates from the upper part of Figure 5 $\log K^{Zn}_{Zn_2ATP} \simeq 3.0$. This value is in reasonable accord with the stability of Ni_2ATP, which in turn agrees well with the results of the middle part of Figure 5, in which precipitation occurred at just about the $[Ni^{2+}]$ for which saturation of the rate should be observed. From the upper part of Figure 4 it follows that Cu_2ATP is rather stable, the lower limit of the stability being $\log K^{Cu}_{Cu_2ATP} > 3.4$, which is in accord with the rough guess[34] $\log K^{Cu}_{Cu_2ATP} \simeq 4.2$.

Dephosphorylation of ATP Complexes Studied by the Method of Continuous Variation. To obtain a clearer picture of the composition of the reactive complex, Job's method[35] was used; the initial rates, v_0, were measured and the results plotted vs. the ratios $[M^{2+}]/([M^{2+}] + [ATP])$, keeping $[M^{2+}] + [ATP]$ constant. Hence, a maximal rate at values of 0.33, 0.5, or 0.67 indicates a composition for the reactive species of M^{2+}:ATP = 1:2, 1:1, or 2:1, respectively.

In a corresponding experiment with the Cu^{2+}-ATP system at pH 5 Schneider and Brintzinger[13] observed a maximum at Cu^{2+}:ATP = 1.2:1, which they took as evidence that the reactive species is a 1:1 complex. However, the experiments described above suggest that the composition of the most reactive species varies with pH. Therefore, experiments were carried out from pH 4.5 to 6.7 (Figure 6). It is clear that the most reactive species is a Cu^{2+}-ATP 2:1 complex at pH 4.5 and a 1:1 complex at pH 6.7.

It would not be expected from the results of Figure 2 that a 1:1 complex with Zn^{2+} or Ni^{2+} is the most reactive species at any pH; thus, for Ni^{2+} we performed the experiments at the highest pH possible without too much interference from precipitation; for Zn^{2+} pH values close to the optima (cf. Figure 2) were chosen. The corresponding Job's series for the Zn^{2+}- and Ni^{2+}-ATP systems is plotted in Figure 7, which shows clearly that under all conditions a complex with the composition M^{2+}:ATP = 2:1 is the most reactive species.

Although the descending sides of the optima (Figures 6 and 7) with higher ratios of $[M^{2+}]/([M^{2+}] + [ATP])$ are easily explained by the decreasing concentration of the reactive complex, the explanation of the data at lower ratios is less clear. For the Ni^{2+} and Zn^{2+} systems, the obvious explanation is that

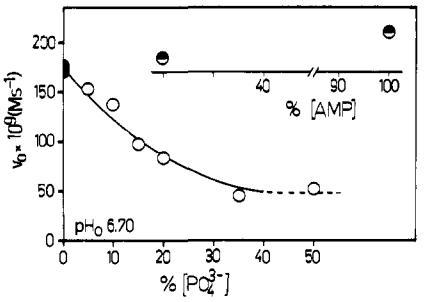


Figure 8. Influence of phosphate or AMP on the dephosphorylation of the Cu^{2+}–ATP system. Dependence of v_0 on the percentage (based on $[ATP]_{tot}$) of KH_2PO_4 or Na_2AMP present: $[Cu^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M; $I = 0.1$, $NaClO_4$; 50 °C. The dashed line indicates uncertainty due to the added high $[PO_4^{3-}]$, which precludes an exact rate determination.

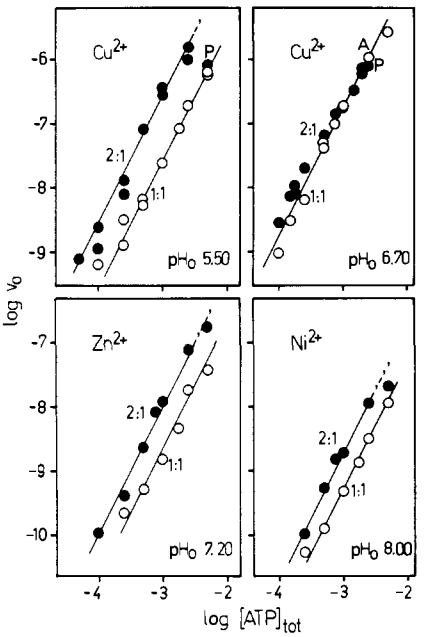


Figure 9. Relationship between dephosphorylation rate of ATP and total concentrations of M^{2+} and ATP. Dependence of v_0 (Ms^{-1}) on $[M^{2+}]_{tot} = 2[ATP]_{tot}$ (●) or $[ATP]_{tot} = [M^{2+}]_{tot}$ (○) for Cu^{2+}, Zn^{2+}, and Ni^{2+} at different values of pH_0 ($I = 0.1$, $NaClO_4$; 50 °C). In the experiments labeled P, precipitation was observed; the dashed lines indicate also uncertainty due to precipitation. The point labeled A results from the evaluation at pH_0 6.70 in part B of Figure 1.

the 1:1 complex is also reactive, but less so than the 2:1 complex (this agrees with the results of Figure 2), and that in addition some $M(ATP)_2^{6-}$ with a low dephosphorylation tendency is formed. However, for the Cu^{2+} system, this alone cannot explain the sharp decrease in rate between the ratios 0.5–0.4 observed in the lower part of Figure 6 (nor that observed in Figure 4). Even if one assumes, as Schneider and Brintzinger did,[13] that any ATP present in excess coordinates to the 1:1 complex and forms $Cu(ATP)_2^{6-}$, i.e., that (i) the stability of this complex is large and (ii) it is not dephosphorylated, one cannot explain the sharp decrease in the rate. The straight line ("T") in the lowest part of Figure 6 is calculated on this basis; the observed rate is still considerably smaller than the expected one.

Another way to explain this observation is inhibition of the reaction by a ligand not yet considered. Indeed, ATP contains about 2–3% PO_4^{3-} already at the beginning,[1] in addition to traces of AMP and ADP. Although ADP is also dephosphorylated (at a lower rate[23]), any possible influence is minimized by the determination of the initial rate v_0; inhibition by AMP and especially PO_4^{3-} appears feasible. Therefore, the experiments of Figure 8 were made; AMP does not inhibit at all, and the inhibition by PO_4^{3-} is too small to explain the discrepancy discussed in the last paragraph.

Hence, there is only one explanation for the sharp decrease in the rates observed between the ratios 0.5 and 0.4 in the lower parts of Figure 6 (cf. also Figure 4). If the 1:1 complex $Cu(ATP)^{2-}$ were the reactive one or a simple isomer of it, the rate would be directly proportional to $[Cu(ATP)^{2-}]$, but the observations could be explained by a species whose concentration depends on $[Cu(ATP)^{2-}]^2$. Thus, the experiments presented in the next section were performed.

Relation between the Rate of Dephosphorylation and the Concentration of M^{2+}–ATP in the 1:1 and 2:1 Systems. The experiments of Figure 9, where log v_0 is plotted vs. log [ATP], were carried out to learn more about the dependence of the rate v_0 on the concentration of $M(ATP)^{2-}$ and $M_2(ATP)$.[36] With M^{2+}:ATP = 1:1, $M(ATP)^{2-}$ is formed to a very large extent (cf. Figure 3); the same holds for the ratio 2:1 and $Cu_2(ATP)$. The degree of formation of $Zn_2(ATP)$ and $Ni_2(ATP)$ is less, but in these cases the reactivity is dominated by the 2:1 species; hence, the desired information may also be obtained.

For the Cu^{2+}–ATP 2:1 and 1:1 systems at pH_0 5.5 and 6.7, straight lines with a slope of two are observed (Figure 9, upper parts); the difference of about one log unit between the lines at pH_0 5.5 corresponds to the rate difference in Figure 2. The same holds for the data at pH_0 6.7, which fit on one straight line for both systems. Slopes of two are also observed for the Zn^{2+} and Ni^{2+} systems at pH_0 7.2 and 8.0, respectively (Figure 9, lower parts). Hence, in all cases the rate depends on the square of the reactant concentration.

This can be rationalized with the monomer–dimer equilibria 1 and 2.

$$2M(ATP)^{2-} \rightleftharpoons [M(ATP)]_2^{4-} \quad (1)$$

$$2M_2(ATP) \rightleftharpoons [M_2(ATP)]_2 \quad (2)$$

Assuming they are far to the left,[37] i.e., the concentrations of the dimers are very small, then their concentration is proportional to the square of the total concentration because $K_D = [dimer]/([monomer])^2$. Hence, if the dimers are the reactive complexes, the slopes of the plots, log v_0 vs. log $[reactant]_{tot}$, must be two, while for the monomer as the reactive species it would be one. Thus, the results of Figure 9 indicate that $[M(ATP)]_2^{4-}$ and $[M_2(ATP)]_2$ are the reactive complexes in the metal ion promoted dephosphorylation of ATP. Stacking with the purine moiety is known,[20-22] as is the self-association of nucleosides[38] and nucleotides, e.g., of ATP.[39] The latter interactions are weak with formation constants[38,39] of the order of 10; there is also evidence that metal ion–adenine nucleotide complexes may form base-stacked adducts.[27,40-43]

Metal Ion Promoted Dephosphorylation of CTP. There is little or no metal ion–cytosine interaction in cytidine nucleotides,[21,27,44-48] and M^{2+}–CTP systems are rather stable toward dephosphorylation, which occurs only to a small extent at alkaline pH.[1,23,24] This suggests a different reaction pathway from the one operating with the purine nucleotides. The first-order rate constants k are plotted vs. pH in Figure 10 for the Cu^{2+}–CTP 1:1 and 2:1 systems. The data determined by Schneider and Brintzinger[13] for the Cu^{2+}–methyltriphosphate 1.1:1 system in the pH range 4–7 and their single point for the Cu^{2+}–MTP 2:1 system are also inserted. The agreement between the Cu^{2+}-promoted dephosphorylation behavior of MTP and CTP both in the 2:1 and in the 1:1 systems is evident and in agreement with the absence of a Cu^{2+}–base interaction in the latter system.

It also follows from Figure 10 that for Cu^{2+}–CTP the 2:1 system is more reactive than the 1:1 system, although homogeneous solutions could only be studied below pH ~5. Complex

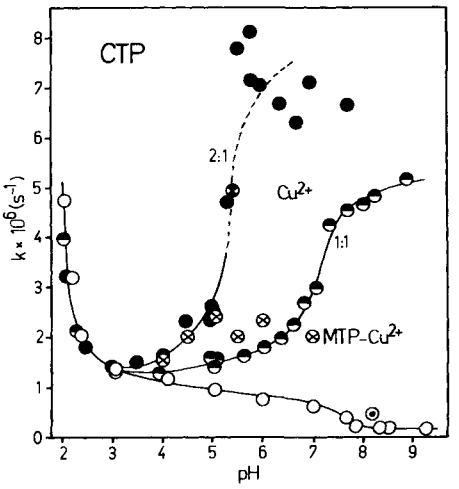


Figure 10. First-order rate constant, k (s^{-1}), for the dephosphorylation of CTP: CTP alone (○),[24] Cu^{2+}–CTP (1:1) (◓),[24] and Cu^{2+}–CTP (2:1) (●). $[CTP]_{tot} = 10^{-3}$ M; $I = 0.1$, $NaClO_4$; 50 °C. The dashed line portion indicates uncertainty due to precipitation. For comparison, the data of methyltriphosphate[13] are also shown: MTP alone (⊙), Cu^{2+}–MTP (1.1:1) (⊗), and Cu^{2+}–MTP (2:1) (⊛). $[MTP]_{tot} = 10^{-3}$ M; 50 °C.

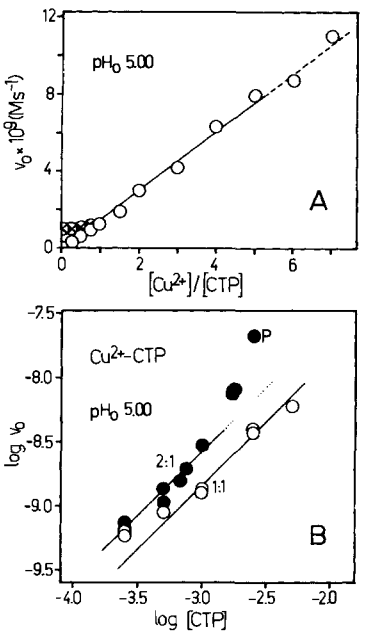


Figure 11. Experiments with CTP at pH_0 5.00 and $I = 0.1$, $NaClO_4$; 50 °C. Part A: dependence of v_0 of the Cu^{2+}-promoted dephosphorylation on $[Cu^{2+}]/[CTP]$ where $[CTP]_{tot} = 10^{-3}$ M (the dashed line indicates uncertainty due to precipitation). The measured points (⊗) were corrected for the dephosphorylation of uncomplexed CTP by assuming 1:1 complex formation.[29] Part B: relation between the dephosphorylation rate of CTP and the total concentrations of Cu^{2+} and CTP. Dependence of v_0 (Ms^{-1}) on $[Cu^{2+}]_{tot} = 2[CTP]_{tot}$ (●) or $[CTP]_{tot} = [Cu^{2+}]_{tot}$ (○). In the experiment of the point labeled P, precipitation was observed. The dotted line portion is tentatively drawn.

formation of CTP in 1:1 systems resembles that of ATP,[21,23,24] and the distribution of complexes shown in the upper part of Figure 3 may be compared to a first approximation with the reactivity of the 1:1 system in Figure 10. It is clear that the increasing reactivity parallels the increasing [Cu(CTP)$(OH)^{3-}$].[24] Unfortunately, all experiments with an excess of Cu^{2+} had to be done at pH_0 5.0, and thus these results apply only to the simple Cu^{2+}–CTP system and we can draw no conclusions about the reactivity of the hydroxo complex.

Figure 11A shows the dependence of the dephosphorylation of CTP on increasing $[Cu^{2+}]$ at pH_0 5.0. The corresponding

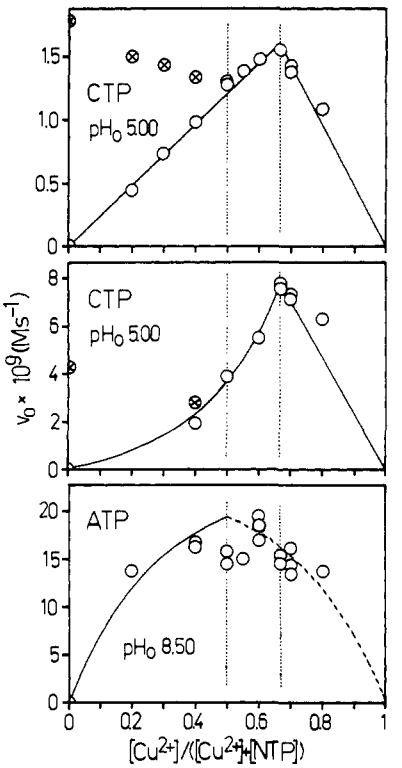


Figure 12. Job's series of Cu^{2+}–CTP and –ATP systems at different values of pH_0; $I = 0.1$, $NaClO_4$; 50 °C. The measured points (⊗) were corrected for the dephosphorylation of uncomplexed CTP by assuming 1:1 complex formation.[29] The dashed line portion indicates uncertainty due to precipitation. Upper and lower parts: $[Cu^{2+}]_{tot} + [NTP]_{tot} = 2 \times 10^{-3}$ M. Middle part: $[Cu^{2+}]_{tot} + [CTP]_{tot} = 5 \times 10^{-3}$ M.

results of the Cu^{2+}–ATP system at pH_0 5.5 (Figure 4, upper part) show a sharp break at a twofold excess of Cu^{2+} and the absence of a similar saturation rate in Figure 11A is surprising. As the formation degrees of $Cu(CTP)^{2-}$ and $Cu(ATP)^{2-}$ at pH 5.0 and 5.5 are comparable,[21,23,24] $Cu_2(CTP)$ must be somewhat less stable than $Cu_2(ATP)$. In 1:1 M^{2+}–nucleotide complexes a possible metal ion–nucleic base interaction is not apparent from the stability constants,[49-53] e.g., the stabilities[54] of $Cu(ATP)^{2-}$ and Cu(methyltriphosphate)$^{2-}$ are very similar. $M_2(NTP)$ seems to be different in this respect. As the phosphate backbone is identical in ATP and CTP, the increased stability of $Cu_2(ATP)$ may indicate that one Cu^{2+} is also bound to the base while this additional interaction is missing in $Cu_2(CTP)$ leading to its lower stability.

The Job's series in Figure 12 shows that the most reactive species contains Cu^{2+} and CTP in the ratio 2:1. Two experiments were carried out under different conditions, because each experiment by itself is not entirely satisfactory. At low concentrations (upper part) the differences in rate between the ratios of 0.5 and 0.67 are not very significant, while at higher concentrations (middle part of Figure 12) the experimental conditions are close to those under which a visible precipitate is formed (cf. Figures 10 and 11B).

In the Cu^{2+}–ATP and Cu^{2+}–CTP 2:1 systems precipitation occurs at higher pH. Despite this, Job's series were carried out at pH_0 8.5, and the one for ATP is given in the lowest part of Figure 12. Although no reliable evidence can be obtained we tentatively conclude that the most reactive species contains M^{2+} and NTP in the ratio 1:1, i.e., any second metal ion is hydroxylated and released. This is supported by the data of Figure 2; at higher pH the 2:1 system approaches the reactivity of the 1:1 system for all three metal ions.

The relationship between the rate of dephosphorylation and the concentration of Cu^{2+}–CTP in the 1:1 and 2:1 systems was

determined. Within experimental error the data fit straight lines with slopes of one (Figure 11B). This indicates that the reaction proceeds in these systems via a monomeric species, i.e., that the initial rate v_0 is directly proportional to $Cu(CTP)^{2-}$ or $Cu_2(CTP)$.

There is a further interesting effect indicated in Figure 11B. In a solution of the 2:1 system with [CTP] = 2.5×10^{-3} M and [Cu^{2+}] = 5×10^{-3} M a precipitate forms; yet the rate is increased compared with the homogeneous system (cf. also Figure 10). The aggregates formed are apparently more susceptible to dephosphorylation than the dissolved complexes. This was only observed in the Cu^{2+}–CTP system.

Discussion

Based on our present and earlier results[1,23,24] of the metal ion promoted dephosphorylation of nucleoside 5′-triphosphates three categories of reactive species are evident: (i) the very effective reaction pathway via dimers, which operates for purine nucleotides, and contrasts with (ii) the very low reactivity of the monomeric M^{2+}–CTP complexes; for all nucleotides studied in alkaline solution (iii) the ternary hydroxo-M^{2+}–NTP complexes of rather low reactivity are important.[1,24] These three pathways and the structures of their reactive complexes will now be discussed more in detail.

Dephosphorylation via Dimeric Purine Nucleotide–Metal Ion Complexes. It was concluded from the results in Figure 9 that dephosphorylation proceeds via a dimer both in the 1:1 and in the 2:1 M^{2+}–ATP systems. As the rate of the Cu^{2+}–ATP 2:1 system coincides at pH 6.5 with that of the 1:1 system (Figure 2), both dimers must be reactive complexes, though the M^{2+}–ATP 2:1 dimers are more reactive at pH 5.3. The whole process may be summarized as in eq 3.

$$2\ M^{2+} + 2\ ATP^{4-} \rightleftharpoons 2\ M(ATP)^{2-} \rightleftharpoons [M(ATP)]_2^{4-} \longrightarrow PO_4^{3-}, \text{etc.} \quad (3a)$$
$$\downarrow\uparrow 2\ M^{2+} \qquad \downarrow\uparrow 2\ M^{2+}$$
$$2\ M_2(ATP) \rightleftharpoons [M_2(ATP)]_2 \longrightarrow PO_4^{3-}, \text{etc.} \quad (3b)$$

For the metal ions Cu^{2+}, Zn^{2+}, or Ni^{2+} (and also Mn^{2+}),[1] the complex $M(ATP)^{2-}$ is formed in M^{2+}–ATP mixtures to a high degree above pH 4 or 5 (Figure 3),[1,21-24] while as we have seen the reactive dimer $[M(ATP)]_2^{4-}$ exists only in low concentrations. This is also true for $[M_2(ATP)]_2$; the stabilities for the corresponding monomer $M_2(ATP)$ are more uncertain, but their existence in appreciable amounts in the presence of excess metal ion is definite.[27,30,32,33,40]

In addition, it is necessary to recall the structures of $M(ATP)^{2-}$: $Cu(ATP)^{2-}$ exists as a macrochelate resulting from the coordination of Cu^{2+} to the β- and γ-phosphate groups and to N(7).[13,23,25,28,44,54] Zn^{2+} interacts also with N(7)[25,33,54,55] and the β- and γ-phosphate groups of ATP^{4-},[25] and at least part of $Zn(ATP)^{2-}$ exists in a ring back-bound form.[54] The Mn^{2+}–N(7) interaction is also known,[25,28,30,46] but Mn^{2+} coordinates to the α-, β-, and γ-phosphate groups,[25,28] and 20% of $Mn(ATP)^{2-}$ exists as a macrochelate.[56] Ni^{2+} coordinates also to all three phosphate groups,[28] and $Ni(ATP)^{2-}$ occurs at least in part as a macrochelate,[28,30,45,46,54] though not all questions regarding the extent of the Ni^{2+}–base interaction are yet solved.[27] The main structural difference between these ATP complexes is that Cu^{2+} and Zn^{2+} coordinate only to the β- and γ-phosphate groups, whereas Mn^{2+} and Ni^{2+} coordinate to all three. There is also evidence for Ni_2ATP from spectrophotometric studies[30] and for Zn_2ATP from NMR[33] that a metal ion–base interaction exists;[27] the same may be surmised for Cu_2ATP. It is known that the Cu^{2+}–base interaction in $Cu(GTP)^{2-}$ corresponds about to the one in $Cu(ATP)^{2-}$,[20b] while it is less in $Cu(ITP)^{2-}$.[20a] In $M(NTP\text{-}H)^{3-}$ with N(1) of ITP and GTP deprotonated, the metal ion–base interaction is significant for Ni^{2+}, Cu^{2+}, and Zn^{2+}, and macrochelates are formed.[21]

Thus, in order to devise the structures of the reactive complexes $[M(ATP)]_2^{4-}$ and $[M_2(ATP)]_2$, we must evaluate (i) the metal ion–nucleic base interaction and (ii) the metal ion–phosphate coordination and labilization of the γ group.

(i) **Importance of the Metal Ion–Base Interaction for the M^{2+}-Promoted Dephosphorylation.** To illustrate some of the more important facts a part of the earlier results[23,24] is summarized in Figure 13. The following points constitute evidence for the dependence of an enhanced dephosphorylation rate on the M^{2+}–base coordination.

(a) In the ternary complex $Cu(Bipy)(ATP)^{2-}$ Cu^{2+}–N(7) coordination is prevented[31] by intramolecular stacking between the pyridyl and purine moieties,[19,20a] and the nucleotide is protected from dephosphorylation (Figure 13).[23] This has also been observed with Cu(imidazole)(ATP).[23]

(b) With increasing concentration of hydroxo complexes, e.g., $Cu(ATP)(OH)^{3-}$, in which the N(7) interaction is inhibited,[20,23] the reactivity of the Cu^{2+}–ATP system decreases (cf. Figures 3 and 13).[23]

(c) The metal ion–base interaction is insignificant in CTP complexes and nonexistent in $Cu(MTP)^{2-}$, and indeed the reactivities of Cu^{2+}–CTP and Cu^{2+}–MTP are very low compared with that of Cu^{2+}–ATP (cf. Figures 10 and 13),[24] although the degrees of complex formation are similar.[24,54] Here a further conclusion must be added. If stacking were the primary task of the base moieties (cf. ref 15 and 16), it is difficult to see why the Cu^{2+}–ATP system should be so different from the Cu^{2+}–CTP system as the cytidine moiety is also able to undergo stacking,[38,57,58] i.e., only the Cu^{2+}–MTP system should be unreactive.

(d) The Cu^{2+}–N(7) interaction in the Cu^{2+}–ITP system at pH 5.5 amounts only to about 10% of that observed with Cu^{2+}–ATP,[20a] although the formation degrees of $Cu(NTP)^{2-}$ are comparable.[21] This corresponds with the dephosphorylation rate of Cu^{2+}–ITP at pH 5.5, which is about 10% of the rate for Cu^{2+}–ATP at this pH (cf. Figure 13).[24] The stacking tendencies of the base moieties of ATP and ITP are similar.[19,20a] However, the coordination tendencies of N(7) of ATP and GTP for Cu^{2+} at pH 5.5 are comparable,[20b] and the dephosphorylation rates of these systems at this pH differ only by a factor of two (Figure 13).[24]

(e) There is a second pH optimum (pH 8.2) in the reactivity patterns of Cu^{2+}–ITP and of Cu^{2+}–GTP (Figure 13) which coincides with the maximum of the formation of Cu(NTP-H);[24] in these species the ring back-binding is strong[21] and occurs either to the N(1),O(6) site or to both the N(7)- and N(1),O(6) sites.[20] The low reactivity of the corresponding ternary systems (Figure 13), in which $Cu(Bipy)(NTP)^{2-}$ and $Cu(Bipy)(NTP\text{-}H)^{3-}$ are formed, and of the hydroxo complexes is again in accord with the inhibited base coordination.[20,21]

(f) The Zn^{2+}–N(7) interaction in the ATP system[54] is less than with Cu^{2+}, and Zn^{2+}–ATP is indeed less reactive (cf. Figure 2), whereas in the NTP-H systems the interaction is more comparable[21] as is the rate of dephosphorylation.[1] However, the overall behavior of the Zn^{2+} systems[1] resembles that of the Cu^{2+} systems. Ternary hydroxo or 2,2′-bipyridyl-containing complexes are also unreactive.

(ii) **Metal Ion–Phosphate Coordination and the Labilization of the γ-Phosphate Group.** The evidence that structural features, other than the M^{2+}–nucleic base interaction, are necessary for enhanced reactivity is as follows:

(a) The Mn^{2+}–ATP system is only slowly dephosphorylated,[1] despite the known interaction between Mn^{2+} and N(7). The only obvious structural difference is that Mn^{2+} coordinates to all three phosphate groups, whereas Cu^{2+} and Zn^{2+} do so only to the β and γ groups.[25,28]

(b) Ni^{2+} also coordinates to all three phosphate groups,[28] and despite the known Ni^{2+}–base interaction and the dimer

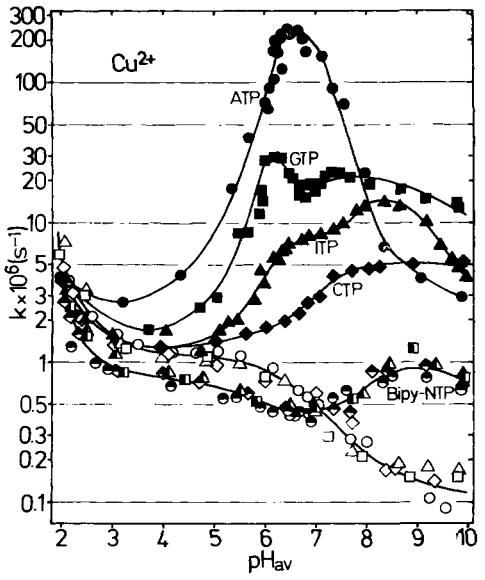


Figure 13. Comparison of the Cu^{2+}-promoted dephosphorylation of ATP, GTP, ITP, and CTP in independence on pH, characterized as the first-order rate constant $k(s^{-1})$: ATP (○),[23] GTP (□, ITP (△), CTP (◇) alone,[24] ATP (●),[23] GTP (■), ITP (▲), or CTP (◆) in the presence of Cu^{2+} (1:1);[24] and ATP (◓),[23] GTP (◫), ITP (◭), or CTP (◈)[24] in the presence of Cu^{2+} and 2,2'-bipyridyl (1:1:1). The concentration of all reagents was 10^{-3} M; $I = 0.1$, $NaClO_4$; 50°C.

formation (cf. Figure 9) the Ni^{2+}-ATP system is not readily dephosphorylated (cf. Figure 2).[1] We thus conclude that coordination to the α-, β-, and γ-phosphate groups causes the ineffectiveness of Ni^{2+} in the metal ion promoted dephosphorylation of ATP. Indeed, the presence of a strong Ni^{2+}-base interaction in the N(1)-deprotonated ITP and GTP systems,[21] or its absence in Ni^{2+}-CTP,[27,40,45,46] has no influence on the reactivity; all Ni^{2+}-NTP systems are dephosphorylated with the same rate.[1]

(c) The structure-reactivity relationship outlined may be rationalized using the hypothesis[13] that in the active complex the metal ion should be coordinated to the β-phosphoryl group or[4,9,12] to the α and β groups.[13] We favor the latter which would have a lower activation energy. We thus conclude that for Cu^{2+} and Zn^{2+} which coordinate to the base moiety, a shift of the metal ion from the β,γ positions along the phosphate backbone to an α,β coordination is initiated, resulting in the dephosphorylation of a γ group in the dimers $[M(NTP)]_2^{4-}$.

(d) An analogous shift could not be initiated in the dimers of Ni^{2+} (and Mn^{2+}) because these cations are coordinated to all three phosphate groups.

(e) Addition of a second M^{2+}, e.g., Ni^{2+}, which coordinates to the more basic γ group forming $[Ni_2(NTP)]_2$ will force an α,β coordination of the first Ni^{2+}, thus enhancing the rate of dephosphorylation. The coordination of a second Zn^{2+} or Cu^{2+} to the corresponding dimers will enhance the reactivity even further because this coordination does not cause a reduction of the number of ligand groups coordinated to the first M^{2+}. On this basis the relatively rate-effective coordination of Ni^{2+} (or Zn^{2+}) to $[Cu(ATP)]_2^{4-}$ leading to the "mixed" dimers $[NiCu(ATP)]_2$ which are even more reactive than $[Cu(ATP)]_2^{2+}$ can also be understood (lower part of Figure 5). The coordination of a second metal ion to the γ group will also enhance the electrophilicity of the phosphorus atom and thus favor a nucleophilic attack.

(f) In the activated dimers $[M(NTP)]_2^{4-}$ or $[M_2(NTP)]_2$, the γ-phosphate group is susceptible to a nucleophilic attack by OH^- or H_2O (either is possible),[1,59] thus leading in the rate-determining step[60] to a pentacovalent phosphorus intermediate,[3,61,62] the formation[3] of which may be favored by the coordination of the second metal ion. ^{18}O incorporation from water into PO_4^{3-} derived from the γ-phosphate group occurs during the Cu^{2+}-promoted dephosphorylation of ATP[63] and in an enzymatic cleavage of the terminal P-O-P bond in GTP.[64]

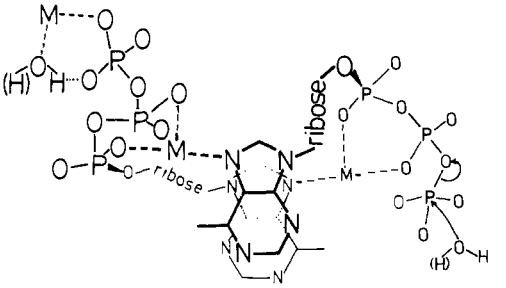


Figure 14. Tentative and simplified structure of the reactive dimer, which occurs in low concentrations during the metal ion promoted dephosphorylation of purine nucleoside 5'-triphosphates.[69] The right side of the structure represents the situation in $[M(NTP)]_2^{4-}$ (cf. eq 3a) and the left side the one in $[M_2(NTP)]_2$ (cf. eq 3b and text).

(g) The relatively low reactivity of the hydroxo complexes $M(NTP)(OH)^{3-}$ and $M(NTP\text{-}H)(OH)^{4-}$ suggests that nucleophilic attack of OH^- (or H_2O) at a terminal phosphate group in the dimer $[M(NTP)]_2^{4-}$ probably occurs "trans" to the α,β-coordinated metal ion and not through intramolecular attack by bound OH^- (or H_2O).[1] However, this may be different in the dimer $[M_2(NTP)]_2$, in which a metal ion also coordinates to the terminal phosphate group.

There is considerable evidence for self-association or base stacking in many nucleosides or nucleotides,[38,39,65,66] i.e., for dimer formation. There are also indications that the formation of $M_2(NP)_2$ and $M(NP)_2$ involves base-stacked ligands in the adenine nucleotides.[27,40-43,52,67] Though the kind of metal ion coordination deduced from NMR measurements[43,52] has been questioned,[68] self-stacking is well established. Based on the above evidence the structure shown in Figure 14 can be deduced for the reactive intermediate in the metal ion promoted dephosphorylation of purine nucleoside 5'-triphosphates. The arrangement of the base moieties follows a suggestion made by Berger and Eichhorn[43,52] about the possible structure of $[Cu(AMP)]_2$.[69] We think that the main features shown in Figure 14 hold not only for the complexes of ATP^{4-}, ITP^{4-}, and GTP^{4-} but also for the ones deprotonated at N(1), i.e., $(ITP\text{-}H)^{5-}$ and $(GTP\text{-}H)^{5-}$, where the N(1),O(6) site is also involved in metal ion coordination.[20] For the metal ion promoted dephosphorylation of purine 5'-diphosphates[23,24] a similar reactive dimer may be postulated, i.e., with metal ion coordination to N(7) and the α-phosphoryl group.

It should be recalled that observations like the lower reactivity of the Zn^{2+}-ATP system, compared with the Cu^{2+} one (Figure 2), can be rationalized with Figure 14. The Zn^{2+}-N(7) interaction is less pronounced than that with Cu^{2+},[54] and, hence, it is less effective in shifting Zn^{2+} along the phosphate backbone into α,β coordination. Even the noninhibitory qualities of AMP, compared to the inhibition by phosphate (Figure 8), can then be understood. While phosphate competes for the coordination at Cu^{2+} and thus inhibits the reaction, AMP participates in the formation of the "dimer" by taking the place of one ATP, and the triphosphate in the species $Cu_2(AMP)(ATP)^{2-}$ could also be hydrolyzed in the way already outlined; the existence of a complex $Cu(AMP)(ATP)^{4-}$ has already been postulated.[27,42]

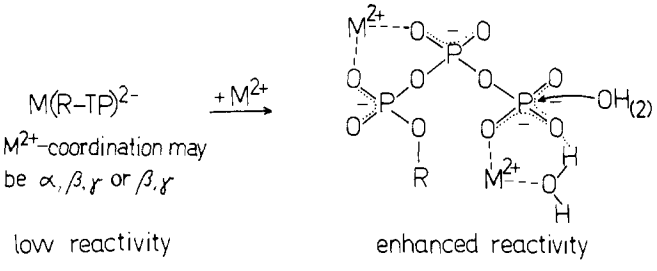
It should be noted that the contradictions mentioned in the introduction are solved with the conclusions summarized in Figure 14 and that a number of features asked for earlier appear in it. Counting only the still valid conclusions and sug-

gestions, we note that (i) Tetas and Lowenstein[12] concluded in 1962–1963 "that the ions which are particularly active in catalyzing the hydrolysis of ATP interact with the adenine ring system". This was reinforced by Schneider and Brintzinger[13] in 1964, when they concluded that the M^{2+}–N(7) interaction is essential, and by Miller and Westheimer[8] in 1965–1966 from a comparison of the rate of dephosphorylation in the M^{2+}–methyl and γ-phenylpropyl triphosphate systems with that of M^{2+}–ATP systems. (ii) Schneider and Brintzinger also concluded that in the active species the metal ion should be coordinated to the β-phosphate group. Tetas and Lowenstein asked for α,β coordination, which was also deduced by Cooperman[4,9] in 1969 from a study of the hydrolysis of unsymmetrical diesters of pyrophosphate. (iii) Miller and Westheimer[8] have suggested that "a complex containing two metal ions per triphosphate may be the reactive species". (iv) In 1967–1968 Spiro et al.[14] concluded that the reactive species is a dimer, a suggestion which was taken up in 1972 by Feldman[15,16] in his theory on "metal ion catalysis of ATP dephosphorylation", who concluded that in the dimer "the two adenine rings are stacked".

Dephosphorylation of CTP and Related Triphosphates in the Presence of M^{2+}. There is little or no cytosine interaction with metal ions in cytidine nucleotide complexes,[21,27,44-48] and the dephosphorylation tendencies of CTP and MTP are indeed very similar (Figure 10). Hence, CTP behaves like a simple organic triphosphate (=R-TP), where metal ions coordinate only to the triphosphate chain.

A comparison of the dephosphorylation rate of the Cu^{2+}–CTP 1:1 system at pH ~5.5 with the rates for free CTP and for the Cu^{2+}–ATP 1:1 system (Figure 13) shows the very low reactivity of the Cu^{2+}–CTP system, i.e., of $Cu(CTP)^{2-}$. The same is observed with Ni^{2+} and Zn^{2+}.[1] The results of Figure 11B indicate that the reactive species is a monomeric complex, the reactivity of which may be enhanced somewhat by the formation of a 2:1 complex, i.e., $Cu_2(CTP)$ (cf. Figures 11A and 12). This may be due to coordination of the second metal ion to the γ group forcing the coordinated metal ion to shift into an α,β position causing labilization of the γ-phosphate group (tentatively indicated in Chart II). The lower reactivity of this

Chart II



species compared with the dimeric ones can be explained by its somewhat lower stability [cf. $Cu_2(CTP)$ with $Cu_2(ATP)$] and, more important, by the absence of the N(7)-facilitated shift of M^{2+} along the phosphate backbone.

Dephosphorylation of Ternary Hydroxo–M^{2+}–NTP Complexes. In the 1:1 systems of CTP and Cu^{2+} or Zn^{2+}, the rate of dephosphorylation increases somewhat with increasing pH, i.e., with increasing $[M(CTP)(OH)^{3-}]$.[1,24] This has also been observed for all of the Ni^{2+}–NTP 1:1 systems.[1] There is no increased reactivity in the pH range 5–8, although $[Ni(NTP)^{2-}]$ reaches about 90%, while a small increase in reactivity at pH about >8.3 is due to the formation of $Ni(ATP)(OH)^{3-}$, $Ni(CTP)(OH)^{3-}$, $Ni(ITP\text{-}H)(OH)^{4-}$, and $Ni(GTP\text{-}H)(OH)^{4-}$. The species $Cu(ATP)(OH)^{3-}$ or $Zn(ATP)(OH)^{3-}$ are considerably less reactive than the corresponding nonhydroxo systems discussed above, though some dephosphorylation still occurs.

Coordination of OH^- to the Cu^{2+}–nucleotide complexes releases the coordinated nucleic base,[20,23] giving hydroxo complexes such as $Cu(CTP)(OH)^{3-}$ or $Cu(ITP\text{-}H)(OH)^{4-}$. Although these are not very reactive they are more sensitive than the free nucleotides and liberation of phosphate probably proceeds via a mechanism which is independent of a metal ion–nucleic base interaction. This may also be true for the hydroxo complexes of the other metal ions. Consistent with this is that the reactivity of any of these metal ions depends little on the nucleotide at higher pH.[1] However, whether or not purine–base stacking occurs under these conditions remains uncertain.

Finally, it appears possible that in these hydroxo complexes an *intra*molecular attack by the metal ion bound OH^- occurs at the terminal phosphate group.[1] That the metal ion is not simply needed for the neutralization of the negatively charged phosphate groups is indicated by the very low reactivity of $M(CTP)^{2-}$, $M(Bipy)(NTP)^{2-}$, and $M(Bipy)(NTP\text{-}H)^{3-}$. A corresponding intramolecular attack of OH^- was recently described by Breslow et al.[71] for the Zn^{2+}-catalyzed hydrolysis of an anhydride, and this conclusion has also been confirmed for other metal ion systems by Buckingham and Engelhardt.[72]

General Conclusions

The close relationship between structure and reactivity is clear from the different sensitivities to dephosphorylation observed in the different M^{2+}–NTP systems. At present, it appears to us that the key part in obtaining a labile γ-phosphate group is the coordination of the metal ion to the α- and β-phosphate groups of a nucleoside triphosphate. In the in vitro studies discussed here this goal is achieved by the formation of dimers (Figure 14) which may be considered as internal ("enzyme")–M^{2+}–(substrate/M^{2+}) complexes. In these, one part of the dimer causes stacking and M^{2+}–N(7) interaction positioning the other NTP so that α,β coordination to the activating metal ion becomes feasible. In vivo a similar mechanism may operate, in which the nucleotide would be fixed by stacking between the base moiety and a suitable aromatic group of the enzyme into a position, such that other ligand groups at the enzyme force the metal ion into α,β coordination at the nucleotide, creating the active complex; this is simplifiedly outlined in Figure 15, part A.

The use of Mg^{2+} in Figure 15 as activating metal ion is deliberate. Bearing the above conclusions in mind, the ideal activating divalent cation should be one which coordinates initially only to the β- and γ-phosphate groups, thus allowing a shift along the phosphate backbone into the α,β coordination without the removal of a binding site, a condition which is fulfilled by Mg^{2+}.[25,52,73] Secondly, this metal ion should have a low tendency to interact with the base moiety of a NTP to prevent the formation of the described "self-reactive" dimers (Figure 14) during the transport of the M^{2+}–substrate complex, again a property of Mg^{2+}.[21,25,55] Even in vitro these structural features manifest themselves and Mg^{2+} is ineffective in promoting the hydrolysis of the terminal phosphate group of ATP.[12,13]

One approach obviously used by nature to overcome transport problems is that described. However, another, more complicated approach is also possible which may occur for metal ions like Zn^{2+}, which show an enhanced dephosphorylation rate in vitro. Here the stabilization of NTP could be achieved through the formation[74,75] of ternary complexes, which are known to be unreactive for imidazole[23] or 2,2′-bipyridyl,[1,23,24] and which certainly exist for amino acids.[22] As there is evidence, e.g., that metal ions are involved in nucleotide-transport processes across membranes,[76] one is tempted to suggest that these proceed via mixed-ligand complexes.

The other suggestion shown in Figure 15, part A, that the base moiety may act as an anchor to fix the nucleotide by stacking to a particular part of the enzyme "surface" is quite feasible as many enzymes have aromatic amino acid residues in their active center. For example, it was suggested that the adenine moitey of ATP is necessary for it to bind to microsomal (Na^{2+} and K^+) ATPase.[77] Although such stacking adducts are usually weak they may be stabilized by additional polar interactions or by metal ion bridges,[22] Indeed, a new absorbance in the uv-difference spectrum of heavy meromyosin is observed only in the presence of Ca^{2+} or Mg^{2+}. This is interpreted as due to stacking between the purine residue of ATP and a tryptophanyl indole group in the active center of the enzyme.[78] Similarly, a study of the binding tendency between arginine kinase and Mn^{2+}, ADP, or ATP revealed that $Mn(ADP)^-$ and $Mn(ATP)^{2-}$ are more tightly bound to the enzyme than Mn^{2+} or the nucleotides alone;[79] this may also be interpreted in terms of an increased stability of ternary complexes[75,76] and a stacking interaction.[22] Such a cooperative effect is used in Figure 15, part A; the stacking adduct is stabilized by the Mg^{2+} bridge between the two components, and the coordination of Mg^{2+} to the enzyme is also favored by the coordination of Mg^{2+} to NTP which is already base-stacked to the enzyme.

The features of the reactive dimer $[M_2(NTP)]_2$ indicated in Figure 14 suggest another possible mechanism for facilitated phosphoryl transfer. This is the activation of the γ-phosphate group by the coordination of the metal ion to this group only; this means that the metal ion must again be shifted from its initial NTP coordination (cf. part B of Figure 15, where D can be a positively charged side chain of the protein or a mono- or divalent metal ion). The general comments already made hold also for part B, but use is also made of the concept[80] that the metal ion may not only coordinate to one of the leaving groups but also to the nucleophile (=X) bearing remote coordination sites. A model study by Sigman et al.[81] of the Zn^{2+}-catalyzed phosphorylation of 1,10-phenanthroline-2-carbinol by ATP should here also be mentioned.

Based on the mechanisms summarized in Figure 15, the failure of $Cr^{III}ADP$ (cf. ref 82 and 83) and $(Cr^{III}ATP)^-$ (cf. ref 83) to serve as substrates for various kinases is explicable; Cr^{III} complexes are kinetically inert, i.e., the ligand-exchange rates are very slow, and a shift along the phosphate backbone as required for the reaction paths outlined is not possible. In fact, this observation could be taken as further support for the mechanisms suggested.

To conclude, there is evidence that both of the possibilities suggested in Figure 15 for the activation of a nucleoside 5'-triphosphate for the transfer of a phosphoryl (or nucleotidyl) group are used in nature. Creatine kinase catalyses the phosphoryl transfer from ATP to creatine and maximal turnover numbers are obtained with Mg^{2+}. For the active complex a structure has emerged[84-86] in which the metal ion is coordinated to the α,β position of ATP as well as to the enzyme but is not bound to the γ group undergoing the transfer or to creatine.[87] On the other hand, the proposed[2,88] structure for the active complex of pyruvate kinase, which catalyzes the phosphoryl transfer from phosphoenol pyruvate to ADP, has a metal ion coordinated to the enzyme, to the terminal phosphate group of ADP, and to the phosphoryl group of phosphoenol pyruvate.

It is difficult to judge at present the importance of direct metal ion–N(7) interactions in these enzyme reactions. There is some evidence for a Mn^{2+}–N(7) interaction in the creatine kinase reaction[86,89] though one wonders how far this is the result of using the paramagnetic Mn^{2+} as a probe in these studies, because the 3d ion Mn^{2+} probably has a somewhat higher affinity[54] for N(7) than the naturally used Mg^{2+} (this is in agreement with the generally observed stability order for ligands with N donors).[50] Finally, it may be noted in this connection that initiation and growth of RNA chains in the DNA-dependent RNA polymerase reaction are metal-ion dependent and usually occur only with ATP or GTP but not with CTP or UTP,[90] i.e., the initiation occurs with those nucleoside 5'-triphosphates which show a significant metal ion–base interaction[20,21] and also[1,24] the strongest metal ion accelerated dephosphorylation.

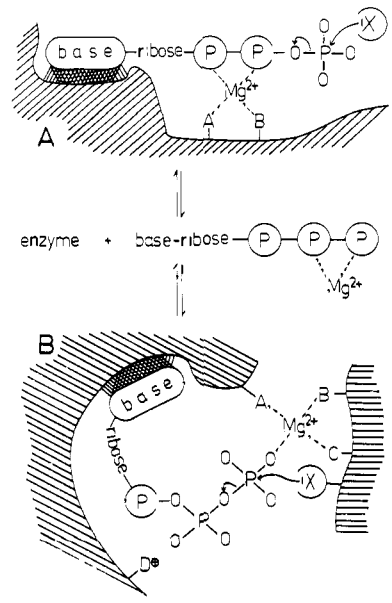


Figure 15. Tentative and simplified structures (A and B) of reactive enzyme–Mg^{2+}–NTP complexes (see text); X represents a nucleophile.

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References and Notes

(1) For part 5, see P. E. Amsler and H. Sigel, *Eur. J. Biochem.*, **63**, 569 (1976).
(2) A. S. Mildvan and C. M. Grisham, *Struct. Bonding (Berlin)*, **20**, 1 (1974).
(3) S. J. Benkovic and K. J. Schray in "Enzymes", Vol. 8, P. D. Boyer, Ed., Academic Press, New York and London, 1973, p 201.
(4) B. S. Cooperman in Vol. 5 of ref 5, 1976, p 79.
(5) H. Sigel, Ed., "Metal Ions in Biological Systems", Marcel Dekker, New York, N.Y.
(6) T. G. Spiro in Vol. 1 of ref 7, 1973, p 549.
(7) G. L. Eichhorn, Ed., "Inorganic Biochemistry", Elsevier, Amsterdam, London and New York, 1973.
(8) D. L. Miller and F. H. Westheimer, *J. Am. Chem. Soc.*, **88**, 1514 (1966).
(9) B. S. Cooperman, *Biochemistry*, **8**, 5005 (1969).
(10) Abbreviations: AMP, ADP, and ATP, adenosine 5'-mono-, -di-, and -triphosphate; Bipy, 2,2'-bipyridyl; CDP, GDP, and IDP or CTP, GTP, and ITP, cytidin,, guanosine, and inosine 5'-di- or -triphosphate, respectively; M, bivalent metal ion; MTP, methyltriphosphate; NTP, nucleoside 5'-triphosphate; NP, nucleotide. The phosphate groups in NTP are labeled as α, β, and γ, where the latter refers to the terminal phosphate group. The formula PO_4^{3-} represents all related species which may be present in a solution, i.e., H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}.
(11) C. Liébecq and M. Jacquemotte-Louis, *Bull. Soc. Chim. Biol.*, **40**, 67, 759 (1958); C. Liébecq, *ibid.*, **41**, 1181 (1959).
(12) M. Tetas and J. M. Lowenstein, *Biochemistry*, **2**, 350 (1963).
(13) P. W. Schneider and H. Brintzinger, *Helv. Chim. Acta*, **47**, 1717 (1964).
(14) T. G. Spiro, W. A. Kjellstrom, M. Zeydel, and R. A. Butow, *Biochemistry*, **7**, 859 (1968).
(15) I. Feldman, *Jerusalem Symp. Quantum Chem. Biochem.* **4**, 528 (1972).

(16) I. Feldman and V. Wee, *Biochemistry*, **13**, 1836 (1974).
(17) S. Suzuki, S. Kimura, T. Higashiyama, and A. Nakahara, *Bioinorg. Chem.*, **3**, 183 (1974).
(18) V. Wee and I. Feldman, Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept 1971, INOR 82.
(19) C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, **96**, 2750 (1974).
(20) (a) C. F. Naumann, B. Prijs, and H. Sigel, *Eur. J. Biochem.*, **41**, 209 (1974); (b) H. Sigel, C. F. Naumann, and B. Prijs, *Ibid.*, **46**, 589 (1974).
(21) H. Sigel, *J. Am. Chem. Soc.*, **97**, 3209 (1975).
(22) H. Sigel and C. F. Naumann, *J. Am. Chem. Soc.*, **98**, 730 (1976).
(23) D. H. Buisson and H. Sigel, *Biochim. Biophys. Acta*, **343**, 45 (1974).
(24) H. Sigel, D. H. Buisson, and B. Prijs, *Bioinorg. Chem.*, **5**, 1 (1975).
(25) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, **237**, 176 (1962).
(26) The difference in temperature between the kinetic data (50 °C) and the equilibrium constants (25 °C) has little influence on the distribution of the species; for more detailed comments see ref 1, 23, and 24.
(27) C. Miller Frey and J. E. Stuehr in Vol. 1 of ref 5, 1974, p 51.
(28) H. Sternlicht, R. G. Shulman, and E. W. Anderson, *J. Chem. Phys.*, **43**, 3123, 3133 (1965).
(29) At the ratios $[M^{2+}]/[NTP] < 1$ the measured rates (⊗, Figure 5) were corrected for the dephosphorylation rate of uncomplexed NTP, assuming (cf. Figure 3) that the 1:1 complexes $M(NTP)^{2-}$ are completely formed. The corresponding corrections were applied in Figures 6, **7**, 11, and 12.
(30) T. A. Glassman, J. Suchy, and C. Cooper, *Biochemistry*, **12**, 2430 (1973).
(31) H. Sigel, K. Becker, and D. B. McCormick, *Biochim. Biophys. Acta*, **148**, 655 (1967).
(32) M. S. Mohan and G. A. Rechnitz, *Arch. Biochem. Biophys.*, **162**, 194 (1974).
(33) T. A. Glassman, C. Cooper, G. P. P. Kuntz, and T. J. Swift, *FEBS Lett.*, **39**, 73 (1974).
(34) $\log K^{Zn}_{ZnATP} - \log K^{Zn}_{Zn_2ATP} = 5.21^{31} - 3.0 = 2.2$; hence, $\log K^{Cu}_{Cu_2ATP} \simeq \log K^{Cu}_{CuATP} - 2.2 = 6.38^{31} - 2.2 = 4.2$.
(35) P. Job, *C. R. Hebd. Seances Acad. Sci.*, **196**, 181 (1933).
(36) The concentration range accessible experimentally is limited: (i) at concentrations $<10^{-4}$ the half-life for dephosphorylation may be several days and more important the small concentrations of phosphate liberated preclude an exact determination; (ii) at high concentrations the reaction can be too rapid and precipitation often occurs.
(37) P. Waldmeier, B. Prijs, and H. Sigel, *Z. Naturforsch., B*, **27**, 95 (1972).
(38) (a) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **89**, 3612 (1967); (b) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *Ibid.*, **90**, 1042 (1968).
(39) M. P. Heyn and R. Bretz, *Biophys. Chem.*, **3**, 35 (1975).
(40) C. Miller Frey and J. E. Stuehr, *J. Am. Chem. Soc.*, **94**, 8898 (1972).
(41) V. Wee, I. Feldman, P. Rose, and S. Gross, *J. Am. Chem. Soc.*, **96**, 103 (1974).
(42) H. Sternlicht, D. E. Jones, and K. Kustin, *J. Am. Chem. Soc.*, **90**, 7110 (1968).
(43) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).
(44) H. Sigel, *Helv. Chim. Acta*, **50**, 582 (1967); *Eur. J. Biochem.*, **3**, 530 (1968).
(45) T. A. Glassman, C. Cooper, L. W. Harrison, and T. J. Swift, *Biochemistry*, **10**, 843 (1971).
(46) G. P. P. Kuntz, T. A. Glassman, C. Cooper, and T. J. Swift, *Biochemistry*, **11**, 538 (1972).
(47) G. Kotowycz, *Can. J. Chem.*, **52**, 924 (1974).
(48) Despite conflicting literature evidence, it is now generally agreed that the metal ion–base interaction in CTP complexes is less than in those of ATP; this is understandable as the purine–N(**7**) binding site is absent in the cytosine moiety.
(49) E. Walaas, *Acta Chem. Scand.*, **12**, 528 (1958).
(50) H. Sigel and D. B. McCormick, *Acc. Chem. Res.*, **3**, 201 (1970).
(51) R. Phillips, S.J., *Chem. Rev.*, **66**, 501 (1966); R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Ibid.*, **71**, 439 (1971).
(52) G. L. Eichhorn in Vol. 2 of ref 7, 1973, p 1191.
(53) A. T. Tu and M. J. Heller in Vol. 1 of ref 5, 1974, p 1.
(54) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).
(55) J. A. Happe and M. Morales, *J. Am. Chem. Soc.*, **88**, 2077 (1966).
(56) Y.-F. Lam, G. P. P. Kuntz, and G. Kotowycz, *J. Am. Chem. Soc.*, **96**, 1834 (1974).
(57) A. Fulton and L. E. Lyons, *Aust. J. Chem.*, **21**, 419 (1968).
(58) T. Montenay-Garestier and C. Hélène, *Biochemistry*, **10**, 300 (1971).
(59) A plot of log K vs. log $[H^+]_{av}$ in the pH range 4–5.5 (cf. Figure 2), where $Cu_2(ATP)$ is presumably almost completely formed, also results in a straight line with a slope of approximately −1, thus indicating that the rate is proportional to $1/[H^+]$;[1,23,24] this may be interpreted as OH^- being the nucleophile.
(60) M. Watanabe, *Bull. Chem. Soc. Jpn.*, **47**, 2048 (1974).
(61) S. J. Benkovic and K. J. Schray, *Biochemistry*, **7**, 4090, 4097 (1968); K. J. Schray and S. J. Benkovic, *J. Am. Chem. Soc.*, **93**, 2522 (1971); J. J. Steffens, I. J. Siewers, and S. J. Benkovic, *Biochemistry*, **14**, 2431 (1975).
(62) F. Ramirez, Y. F. Chaw, J. F. Marecek, and I. Ugi, *J. Am. Chem. Soc.*, **96**, 2429 (1974).
(63) H. Moll, P. W. Schneider, and H. Brintzinger, *Helv. Chim. Acta*, **47**, 1837 (1964).
(64) M. S. Rohrbach, M. E. Dempsey, and J. W. Bodley, *J. Biol. Chem.*, **249**, 5094 (1974).
(65) O. Jardetzky, *Biopolym. Symp.*, **1**, 501 (1964).
(66) P. O. P. Ts'o, J. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963); M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).
(67) R. S. Tobias, G. Y. H. Chu, and H. J. Peresie, *J. Am. Chem. Soc.*, **97**, 5305 (1975).
(68) W. G. Espersen and R. B. Martin, *J. Am. Chem. Soc.*, **98**, 40 (1976).
(69) A structure, in which the imidazole part of one purine nucleotide is located over the pyrimidine ring of the other and vice versa,[67,70] could occur. However, molecular models suggest that simultaneous binding of the metal ion to N(7) of one nucleotide and the α,β-phosphate groups of the other is then only possible if the nucleotides have the syn conformation, whereas the anti conformation is usually preferred.[38b,70]
(70) K. Aoki, *Bull. Chem. Soc. Jpn.*, **48**, 1260 (1975).
(71) R. Breslow, D. E. McClure, R. S. Brown, and J. Eisenach, *J. Am. Chem. Soc.*, **97**, 194 (1975).
(72) D. A. Buckingham and L. M. Engelhardt, *J. Am. Chem. Soc.*, **97**, 5915 (1975).
(73) H. Brintzinger, *Biochim. Biophys. Acta*, **77**, 343 (1963).
(74) H. Sigel in Vol. 2 of ref 5, 1973, p 63.
(75) H. Sigel, *Angew. Chem.*, **87**, 391 (1975); *Angew. Chem., Int. Ed. Engl.*, **14**, 394 (1975).
(76) W. Stillwell and H. C. Winter, *Biochem. Biophys. Res. Commun.*, **56**, 617 (1974).
(77) W. A. Brodsky and A. E. Shamoo, *Biochim. Biophys. Acta*, **291**, 208 (1973).
(78) H. Yoshino, F. Morita, and K. Yagi, *J. Biochem. (Tokyo)*, **71**, 351 (1971); F. Morita, *Biochim. Biophys. Acta*, **343**, 674 (1974).
(79) D. H. Buttlaire and M. Cohn, *J. Biol. Chem.*, **249**, 5733, 5741 (1974).
(80) P. Woolley [*J. Chem. Soc., Chem. Commun.*, 579 (1975)] found that the zinc-ion catalysis of the hydration of acetaldehyde is "markedly increased by coordination of a ligand bearing a remote general base".
(81) D. S. Sigman, G. M. Wahl, and D. J. Creighton, *Biochemistry*, **11**, 2236 (1972).
(82) D. Foster and A. S. Mildvan, *Bioinorg. Chem.*, **1**, 133 (1972).
(83) M. L. DePamphilis and W. W. Cleland, *Biochemistry*, **12**, 3714 (1973).
(84) M. Cohn and J. Reuben, *Acc. Chem. Res.*, **4**, 214 (1971); G. H. Reed and M. Cohn, *J. Biol. Chem.*, **247**, 3073 (1972).
(85) J. J. Villafranca in Vol. 4 of ref 5, 1974, p 29.
(86) R. A. Dwek, R. J. P. Williams, and A. V. Xavier in Vol. 4 of ref 5, 1974, p 61.
(87) A similar suggestion has been made for the mechanism of *E. coli* DNA polymerase I, for which "coordination of the α-phosphoryl group of *d*-TTP by the enzyme–Mn^{2+} complex would increase its susceptibility to nucleophilic attack, thus facilitating nucleotidyl transfer", and "the role of DNA polymerase may well be to shift the coordination of Mn^{2+} to the α-phosphoryl group of the bound deoxynucleoside triphosphate substrate, activating the α-phosphoryl group toward nucleophilic attack and nucleotidyl transfer". Cf. ref 2 and J. P. Slater, I. Tamir, L. A. Loeb, and A. S. Mildvan, *J. Biol. Chem.*, **247**, 6784 (1972).
(88) A. S. Mildvan and M. Cohn, *J. Biol. Chem.*, **241**, 1178 (1966); M. Flashner, I. Tamir, A. S. Mildvan, H. P. Meloche, and M. J. Coon, *ibid.*, **248**, 3419 (1973); T. Nowak, *ibid.*, **248**, 7191 (1973); C.-H. Fung, A. S. Mildvan, A. Allerhand, R. Komoroski, and M. C. Scrutton, *Biochemistry*, **12**, 620 (1973).
(89) A. S. Mildvan and M. Cohn, *Adv. Enzymol.*, **33**, 1 (1970).
(90) U. Maitra and J. Hurwitz, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 815 (1965); U. Maitra, Y. Nakata, and J. Hurwitz, *J. Biol. Chem.*, **242**, 4908 (1967); T. Terao, J. E. Dahlberg, and H. G. Khorana, *J. Biol. Chem.*, **247**, 6157 (1972).