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Metal ion-assisted stacking interactions and the facilitated hydrolysis of nucleoside 5′-triphosphates

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Abstract: The self-association properties of the common nucleosides and nucleotides are summarized; if defined via their nucleobases they decrease in the order, adenine > guanine > hypoxanthine > cytosine ≳ uracil. Next, some aspects of the metal ion-promoted dephosphorylation of nucleoside 5'-triphosphates (NTPs) are reviewed. It is shown that the dephosphorylation rate in the presence of Cu^{2+} decreases in the series, ATP > GTP > ITP > pyrimidine-NTPs. Similarly, addition of AMP, GMP or IMP (decreasing order of effectiveness) to a Cu^{2+}/ATP system facilitates the dephosphorylation reaction further because one of the two ATPs in the stacked reactive intermediate, occurring in low concentration, $[Cu_2(ATP)]_2(OH)^-$, has a structural role and this 'enzyme'-like ATP^{4-} can be replaced by one of the mentioned nucleoside 5'-monophosphates. These results demonstrate how weak interactions, i.e. aromatic-ring stacking, can govern the reactivity of a system. Next to stacking, the purine(N7)-metal ion interaction allowing bridging and stabilization of the stack by inclusion of the phosphate group(s) is important; therefore, addition of tubercidin 5'-monophosphate (= 7-deaza-AMP^{2-}) to Cu^{2+}/ATP *inhibits* the reactivity of the system. The structural delicacy of the reactive intermediate is further emphasized by the inhibiting effects of 1,N^6-ethenoadenosine 5'-monophosphate (ε-AMP^{2-}) and adenosine 5'-monophosphate *N*(1)-oxide; in contrast, the dianion of the antiviral 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$) mimics AMP^{2-} exceedingly well and facilitates even further the Cu^{2+}-promoted dephosphorylation of ATP.

1. SOME BACKGROUND INFORMATION

There are numerous statements in the literature like the following ones: (i) *Noncovalent interactions are essential for the specificity and high efficiency of biological reactions* [1-4];[a] such weak interactions, like hydrogen or electrostatic bonding and hydrophobic or aromatic ring stacking, govern recognition reactions of the kind enzyme-substrate, nucleic acid-protein or neurotransmitter-receptor. (ii) *Nucleotides play a key role in cell metabolism* [5], ATP being especially important [6,7], and their high concentrations in certain cell organelles, like the chromaffin granules of the adrenal medulla [8,9] or the 5-hydroxytryptamin organelles of mammalian blood platelets [9], where nucleotide concentrations (mainly of ATP) of up to about 0.8 M are reached [9], require self-association for osmotic stability (see also the reviewed data in [10]). (iii) *The biological transfer of phosphoryl or nucleotidyl groups occurs in the presence of divalent metal ions* [11-13] and it has been estimated [6], based on known metabolic pathways and the extent of the world's biomass, that ATP, and ADP and inorganic phosphate (P_i) from which it is formed, participate in more chemical reactions than any other compound on the Earth's surface, except water.

As said, statements of the mentioned kind are legion and it is therefore tempting to search for 'simple' *in vitro* examples where the indicated observations, i.e. (i) weak noncovalent interactions, (ii) the involve-

a) The reference numbers are given in square brackets and those of equilibria or reactions in parentheses.
Abbreviations: See Figures 1 and 8. M^{2+}, divalent metal ion; P_i, inorganic phosphate. Species given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context.

ment of nucleotides and (iii) transphosphorylations in the presence of metal ions, are interlinked with each other. A few such examples, (mainly) based on the hydrolysis of nucleoside 5'-triphosphates (NTPs), the transfer of a phosphoryl group to water being the simplest transphosphorylation, will be considered below. However, at first it is necessary to review shortly the most prominent features regarding the self-association of nucleosides and nucleotides.

2. THE SELF-ASSOCIATION PROPERTIES OF NUCLEOSIDES AND NUCLEOTIDES WITH EMPHASIS ON SOME SALIENT FEATURES

Today it is generally accepted that self-association of nucleobases (see Fig. 1 [14-16]) occurs via stacking, that it proceeds beyond the dimer stage and that oligomers are formed [3,17-20]. ^{1}H-NMR shift measurements proved ideal to characterize the self-association of nucleosides and nucleotides (= N) (Fig. 1) in aqueous solution (D_2O) [17,19-21]. The observed upfield shifts of the resonances, especially of the nucleobase protons, in dependence on the increasing concentration of N confirm that the association occurs via stacking of the aromatic residues. In most instances the association process is best quantified by application of the isodesmic model for an indefinite non-cooperative self-assocation [15,17-23]; i.e., all association constants are considered as equal:

$$(N)_n + N \rightleftharpoons (N)_{n+1} \qquad (1a)$$

$$K = [(N)_{n+1}]/([(N)_n][N]) \qquad (1b)$$

The relationship between the observed chemical shift (δ_{obsd}) and a solution with the total concentration [N] is given in equation 2,

$$\delta_{obsd} = \delta_\infty + \{(\delta_\infty - \delta_0)[1 - (4K[N] + 1)^{0.5}]/2K[N]\} \qquad (2)$$

where δ_0 represents the shift at infinite dilution (monomeric N), δ_∞ the shift of a molecule in an infinitely long stack, and K is the association constant (eq. 1). The expression considering only dimer formation is of a form analogous to equation 2 [17,24]; hence, whether a system dimerizes or polymerizes can only be concluded from the extent of the upfield shifts.

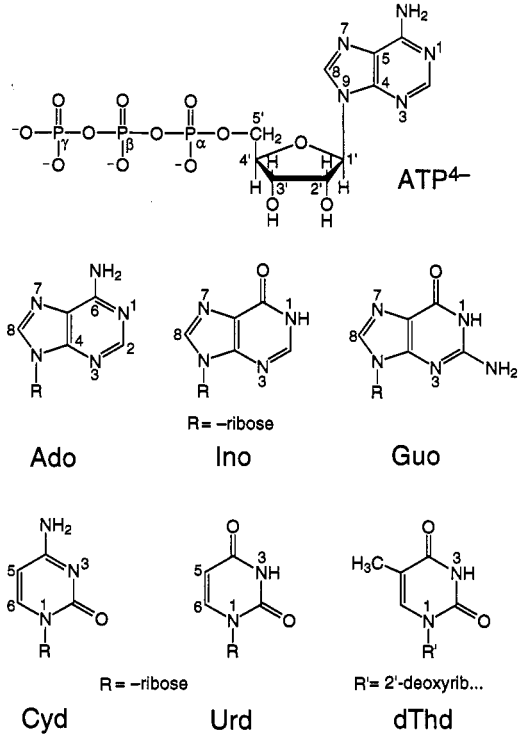


Fig. 1. *Top*: Chemical structure of adenosine 5'-triphosphate (ATP^{4-}) in its predominant *anti* conformation [14-16], together with the labelling system of the triphosphate chain. *Lower part:* Chemical structure of the nucleobases (R = R' = H: adenine, hypoxanthine, guanine, cytosine, uracil and thymine) of the nucleosides (Ns): adenosine (Ado), inosine (Ino), guanosine (Guo), cytidine (Cyd), uridine (Urd) and thymidine (= 1-(2'-deoxy-β-*D*-ribofuranosyl)thymine; dThd). The respective 5'-nucleotides carry a phosphate residue at the 5' end of the ribose moiety: nucleoside 5'-monophosphate (NMP^{2-}), e.g., adenosine 5'-monophosphate (AMP^{2-}); nucleoside 5'-diphosphate (NDP^{3-}), e.g., adenosine 5'-diphosphate (ADP^{3-}); nucleoside 5'-triphosphate (NTP^{4-}), e.g., adenosine 5'-triphosphate (ATP^{4-}); etc.. Replacement of the adenine residue in the structure shown at the top for ATP^{4-} by one of the other nucleobases depicted (if done within the plane) will also result in the *anti* conformation for the corresponding NTP^{4-}.

Some relevant results are summarized in Table 1. From these data it is evident that the selfstacking tendency decreases for the nucleosides (Fig. 1) in the series, Ado > Guo > Ino > Cyd ≳ Urd, reflecting the decreasing aromaticity and hydrophobic properties of their nucleobase residues. A further comparison of the data given in Table 1 shows that the association tendency also decreases in the series, Ns > NMP^{2-} > NDP^{3-} > NTP^{4-}, which is evidently governed by the effect of the increasing negative charge of the phosphate residues. This observation suggests that charge neutralization at the phosphate group of a nucleotide facilitates its self-association.

Indeed, Mg^{2+} coordinated to ATP^{4-} gives $Mg(ATP)^{2-}$ and the self-association constant of this complex (K = 4.0 ± 0.5 M^{-1} [19]) is larger than the one for ATP^{4-} (K = 1.3 ± 0.2 M^{-1} [19]) as expected. However, the

TABLE 1. Association Constants for the Self-Stacking of Several Nucleosides and Some of Their Nucleotides as Determined by ^{1}H-NMR Shift Mesasurements in D_2O (27°C; I = 0.1 or 0.1 – ~2 M, $NaNO_3$)[a]

System defined	K (M^{-1}) (eq. 1)			
via Ns	Ns	NMP^{2-}	NDP^{3-}	NTP^{4-}
Ado	15 ±3[b]	2.1 ± 0.3[b]/1.9[c]	1.8±0.5[d]	1.3 ± 0.2
Guo	8 ±3	1.3[c]	1.0±0.5[d]	0.8 ± 0.6
Ino	3.3 ± 0.3	1.4[c]	1.3±0.6[d]	0.4 ± 0.3
Cyd	1.4 ± 0.5		0.7[d]	0.5 ± 0.2
Urd	1.2 ± 0.5		0.6[d]	~0.4

[a] Average of the results (with *twice* the standard error) obtained from the chemical shifts of H2, H8, and H1' for the purine derivatives and of H5, H6, and H1' (see Fig. 1) for the pyrimidine derivatives. The constants are from [19] if not otherwise indicated. [b] From [22]. [c] From [18]. [d] From [23]; in this ref. also values for the self-association of $M(NDP)^-$ species are given.

association constant for $Mg(ATP)^{2-}$ also considerably exceeds K = 2.1 ± 0.3 M^{-1} [22] for AMP^{2-}, both species being twofold negatively charged. Hence, Mg^{2+} affects the stability of the ATP^{4-} (and ADP^{3-}) stacks beyond a pure charge neutralization, indicating an *inter*molecular bridging by Mg^{2+} (reviewed in [25]). The chemical shift values for δ_∞ give no indication [19,23] for a selective Mg^{2+}-nucleobase interaction in the stacks, suggesting a bridging by Mg^{2+} via the phosphate chains.

For the ATP^{4-} complexes with Zn^{2+} ($K \simeq$11.1 ± 4.5 M^{-1} [19]) or Cd^{2+} ($K \simeq$ 17 M^{-1} [19]) the self-association tendency is much larger than for the mentioned corresponding Mg^{2+} complex. This observation is explained by the formation of an *inter*molecular metal ion bridge in *dimeric* stacks by coordination of Zn^{2+} or Cd^{2+} to the phosphate moiety of one ATP^{4-} and to N7 of the other [19,23,25]. Of course, these relatively stable dimeric stacks, like $[Zn(ATP)]_2^{4-}$, may further associate to larger aggregates in the usual manner. The chemical shifts of the various hydrogens for complete stacking (δ_∞) agree with this interpretation [19,23]. It should be noted that the "constants" given above for the Zn^{2+} and Cd^{2+} systems are based on the isodesmic model (eq. 1) to enable direct comparisons with the other constants given, but they do not describe the experimental results in the best way (for details see [19,23] and the review [25]). For the present the important point is that metal ions like Zn^{2+} or Cd^{2+} favor the formation of dimers by bridging the phosphate *and* N7 sites of the two nucleotides forming the dimer.

How does protonation affect self-association? As can be seen from Table 1, the self-stacking properties of adenosine are quite pronounced (K = 15 ± 3 M^{-1} [22]). If N1 is protonated to 50% the association tendency decreases, i.e., for the $D(Ado)^+$/Ado = 1:1 system one measures K = 6.0 ± 1.3 M^{-1} [22], and for $D(Ado)^+$ the association tendency has nearly vanished (K = 0.9 ± 0.2 M^{-1} [22]). This result is expected because of the repulsion of the positively charged adenine residues; it is also analogous to the observations [26] made with guanosine and inosine where protonation occurs at N7 [26,27]. This simple pattern becomes more complicated if the effect of protonation on the self-association of nucleotides is considered. With AMP a maximum of self-association is observed [22] in dependence on protonation: AMP^{2-} (K = 2.1 ± 0.3 M^{-1}) < $D(AMP)^-$ (K = *3.5* M^{-1}) < $D(AMP)^-/D_2(AMP)^\pm$ = 1:1 (K = 5.6 ± 0.5 M^{-1}) ≫ $D_2(AMP)^\pm$ (K = *1* M^{-1}) > $D_3(AMP)^+$ (K < *0.7* M^{-1}) (the values for K printed in *italics* are intra- or extrapolated from the association constant-pD profile in [22]). This means, neutralization of one of the two negative charges of the $-PO_3^{2-}$ group by protonation leads to a reduced repulsion and thus to a somewhat increased stacking tendency; however, self-stacking is clearly most pronounced if 50% of the adenine residues are protonated at N1, whereas complete base protonation reduces the stacking tendency drastically as is evident from K = 1 M^{-1} for $D_2(AMP)^\pm$.

Though apparently in part somewhat complicated, the mentioned self-association patterns in dependence on pH for adenosine and AMP are easily rationalized and possibly even predictable. This is different for the self-association of ATP in dependence on its protonation degree: ATP^{4-} (K = 1.3 ± 0.2 M^{-1}) < $D(ATP)^{3-}$ (K = 2.1 ± 0.3 M^{-1}) < $D(ATP)^{3-}/D_2(ATP)^{2-}$ = 1:1 (K = 6.0 ± 2.0 M^{-1}) ≪ $D_2(ATP)^{2-}$ (K ≃ 200 M^{-1}) ≫ $D_3(ATP)^-$ (K < 10 M^{-1}) [24]. This result is surprising because the self-association tendency is most pronounced for $D_2(ATP)^{2-}$ which carries a proton each at the terminal γ-phosphate group and at N1. Furthermore, this most pronounced stability is due to a dimeric $[H_2(ATP)]_2^{4-}$ stack as is evident from the size of the observed upfield shift, i.e. $\Delta\delta = \delta_0 - \delta_\infty$ [24], which is compatible with a dimer,

but not with a polymer. This dimer is stabilized by intermolecular ion pair formation between H^+(N1) and the phosphate group and hydrogen bonds between γ-$P(O)_2(OH)^-$ and N7 [24]. This interpretation agrees with the properties of $H_2(GTP)^{2-}$ and $H_2(ITP)^{2-}$ [26]; both species show a low stacking tendency because their *neutral* H(N1) site is not able to form the mentioned *inter*molecular ion pair with the (also present) γ-$P(O)_2(OH)^-$ group, and the proton now located at N7 leads to repulsion of the purine moieties. The above interpretation is further confirmed by a study [28] of the acid-base properties of the purine residues in ITP, GTP, and ATP in dependence on [NTP]: The acidity of the H^+(N7) site in $H_2(ITP)^{2-}$ and $H_2(GTP)^{2-}$ is enhanced upon stack formation due to repulsion of the positive charges, whereas the acidity of the H^+(N1) site in $H_2(ATP)^{2-}$ is *reduced* because this site is also involved in ion pair formation and hydrogen bonding. These observations were mentioned here to emphasize again the preference of ATP to form under certain conditions dimers, like in the present case $[H_2(ATP)]_2^{4-}$.

3. THE METAL ION-PROMOTED DEPHOSPHORYLATION OF PURINE-NUCLEOSIDE TRIPHOSPHATES

The fact that metal ions promote the dephosphorylation of NTPs in aqueous solution according to reaction 3 has long been recognized [29-31].

$$NTP + H_2O \longrightarrow NDP + P_i \qquad (3)$$

Extensive mechanistic studies including Job's series [32], where the initial rates, $v_o = d[P_i]/dt$ (Ms^{-1}), were measured and plotted *versus* the ratios $[M^{2+}]/([M^{2+}]+[ATP])$, keeping $[M^{2+}]+[ATP]$ constant, revealed, like the example shown in Fig. 2 [33], for ATP (and also other NTP) systems that the most reactive species contains two metal ions per ATP (for details see [33-37] and the comprehensive review [12]). Further experiments like those shown in Fig. 3 [33], where log v_o is plotted *versus* log $[ATP]_{tot}$ for several Cd^{2+}/ATP systems, demonstrated that the rate of the dephosphorylation reaction depends on the square of the reactant concentration [12,33,34], indicating that dimers are formed in agreement with ^{1}H-NMR evidence [12]. Indeed, from experiments of the indicated kind one has to conclude [12,33,34] that the reactive species has the composition $[M_2(ATP)]_2$. An even higher reactivity is observed under conditions where one of the two metal ions involved per ATP carries an OH^-, i.e., via the $M(OH)^+$ unit an intramolecular attack at the γ-phosphate group can occur [12,33]. The tentative structure of this most reactive species, $[M_2(ATP)]_2(OH)^-$, is shown in Fig. 4 [12,33,38]. The M^{2+} bridge in the dimer involving N7 is based on various evidence, including ^{1}H-NMR [33] (for further details [12] should be consulted).

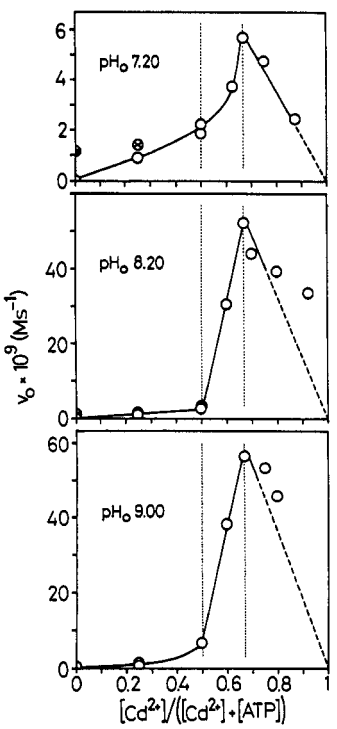


Fig. 2. Job's series for the Cd^{2+}/ATP system at different values of pH_o (= initial pH of the reaction). $[Cd^{2+}]_{tot} + [ATP]_{tot}$ = constant = $2 \cdot 10^{-3}$ M; I = 0.1 M, $NaClO_4$; 50°C. The measured points (⊗) were corrected for the dephosphorylation of uncomplexed ATP by assuming complete 1:1 complex formation. The broken line portions indicate uncertainty due to precipitation. The vertical dotted lines give the positions of the ratios Cd^{2+}:ATP = 1:1 or 2:1. As the maximum rate, expressed as $v_o = d[P_i]/dt$ (Ms^{-1}) [12], is observed at the latter ratio the most reactive species contains two metal ions bound per ATP (for details see Section 6 of [33]). Reproduced by permission of the American Chemical Society from the 'Supplementary Material' (Fig. S1) of [33] (*J. Am. Chem. Soc.*, 1984).

Though the kinetic properties of the M^{2+}/ITP and GTP systems have not yet been studied in full detail, the available results (see Section F in [12]) for the corresponding Cu^{2+}/NTP systems suggest for the reactive intermediate also in these cases the composition $[Cu_2(NTP)]_2(OH)^-$. Evidently, for this reactive intermediate the same structure as the one shown in Fig. 4 can be proposed; in the dimer, ATP^{4-} simply has to be replaced by ITP^{4-} or GTP^{4-}.

With the mentioned conclusions in mind it is interesting to view the rate-pH profiles shown in Fig. 5 for the Cu^{2+} 1:1 systems of ATP, ITP, and GTP as well as of those with CTP, UTP, and dTTP [33]. The second maximum observed in the rate-pH profiles for the Cu^{2+}/GTP and Cu^{2+}/ITP systems is connected with the deprotonation at N1 and not of further concern in the present context. Here I would like to em-

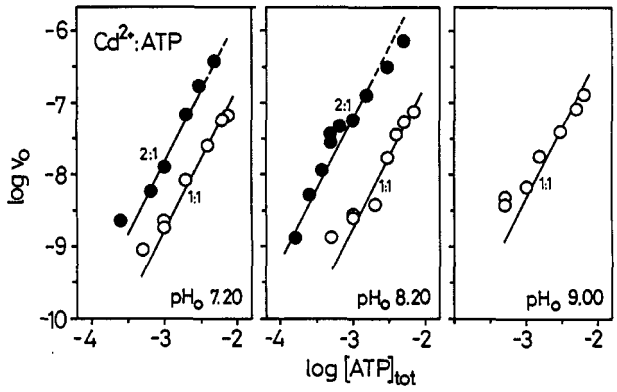


Fig. 3. Relationship between the initial dephosphorylation rate, v_0 (Ms^{-1}), of ATP and the total concentrations of Cd^{2+} and ATP at 2:1 and 1:1 ratios in aqueous solution at different values of pH_0. Dependence of log v_0 on log $[ATP]_{tot}$ = log $(1/2[Cd^{2+}]_{tot})$ (●) or log $[ATP]_{tot}$ = log $[Cd^{2+}]_{tot}$ (○); I = 0.1 M, $NaClO_4$; 50°C. The solid lines are drawn with the slope m = 2 (see [12,33]); the broken line portions indicate the regions of uncertainty due to precipitation. This figure is reproduced with permission of the American Chemical Society from the 'Supplementary Material' (Fig. S2) of [33] (*J. Am. Chem. Soc.*, 1984) by deleting several data points, which are not of relevance in this context (see footnote 62 in [33]).

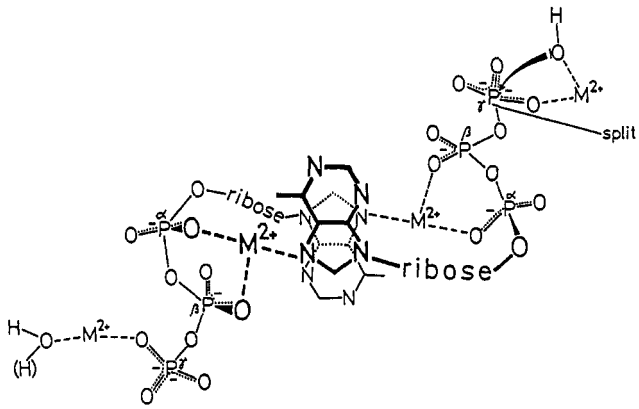


Fig. 4. Proposed structure of the reactive $[M_2(ATP)]_2(OH)^-$ dimer, which occurs in low concentration during the metal ion promoted dephosphorylation of ATP (and other purine-NTPs; see text in Section 3). The intramolecular attack of OH^- is indicated on the right-hand side, while the left-hand side is ready to transfer also into the reactive state by deprotonation of the coordinated water molecule or to undergo an intermolecular water attack (corresponding to the dimeric $[M_2(ATP)]_2$ species).

phasize that Fig. 5 shows that at pH < 6 the reactivity regarding dephosphorylation decreases for the various Cu^{2+} systems in the order, ATP > GTP > ITP > pyrimidine-NTP. This decrease in reactivity observed for the purine-NTPs parallels the decreasing tendency for self-association -- and this of course, affects the formation degree of the reactive dimeric $[Cu_2(purine\text{-}NTP)]_2(OH)^-$ intermediate (Fig. 4). That the decreasing reactivity is connected with the decreasing stacking tendency (see Section 2) and *not* with the affinity of N7 toward metal ions follows from the fact that this affinity shows the reverse order [27]. This comparison supports then the conclusion (see Fig. 4) that stacking interactions are important in forming the kinetically active dimers.

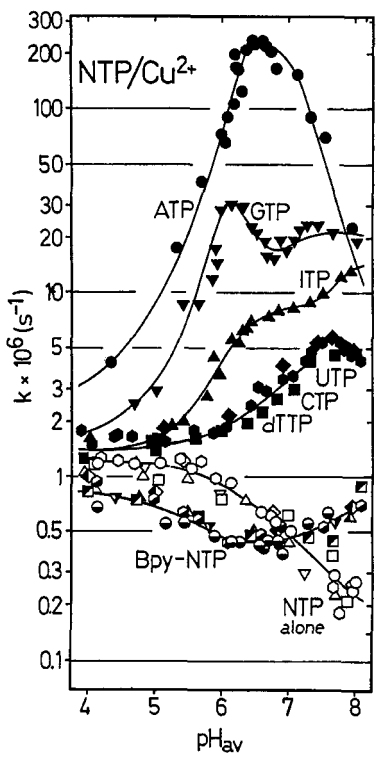
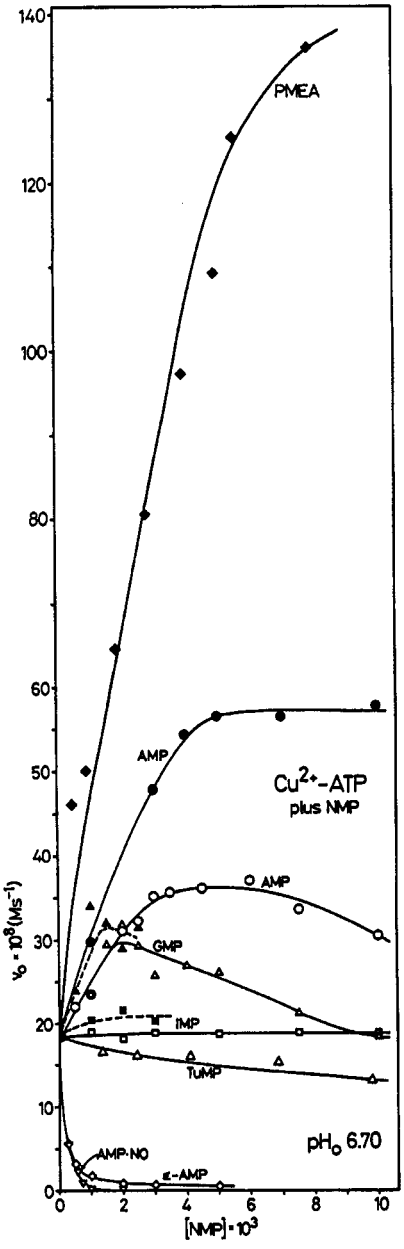


Fig. 5. Comparison of the Cu^{2+}-promoted dephosphorylation of ATP, GTP and ITP as well as of CTP (■), UTP (●) and dTTP (◆) (always in the ratio Cu^{2+}/NTP = 1:1) in aqueous solution as a function of pH, characterized as the first-order rate constants k (s^{-1}) [12]. In addition are shown, but not discussed in the present context, the rate constants for: ATP, GTP, ITP, CTP, UTP and dTTP alone (empty points), as well as the corresponding NTP/Cu^{2+}/2,2'-bipyridyl (Bpy) systems in the ratio 1:1:1 (half filled points). The concentration of each reagent was always 10^{-3} M, when present; I = 0.1 M, $NaClO_4$; 50°C. Partial reproduction of Fig. 1 in [33] (*J. Am. Chem. Soc.*, 1984) by permission of the American Chemical Society.

The Cu^{2+}/pyrimidine-NTP systems in Fig. 5 show the lowest reactivity and in fact, a reactivity which is identical for CTP, UTP, and dTTP, demonstrating that the nucleobase residue has no effect on the reactivity of these systems. Moreover, in accord with the low tendency for self-association detailed mechanistic studies lead to the conclusion that the most reactive species in these systems has the composition M_2(pyrimidine-NTP)$(OH)^-$ [12,35].

4. FURTHER ENHANCEMENT OF THE Cu^{2+}-PROMOTED ATP HYDROLYSIS BY AMP AND EFFECTS OF SOME AMP-RELATED COMPOUNDS

The reactive dimer shown in Fig. 4 may be viewed by concluding that one ATP is needed to bring the other into the reactive state; i.e., one could say ATP serves as its own 'enzyme'. If this is correct one may ask whether the structuring ATP in the dimer cannot be replaced by another ligand. The simplest and most obvious case of such a ligand which still can stack, offers N7, and also owns a phosphate group, is AMP^{2-} (Fig. 1). Indeed, addition of AMP at pH 6.7 to a Cu^{2+}/ATP 1:1 (open symbols) or 2:1 (solid symbols) system further facilitates the dephosphorylation reaction, as can be seen from Fig. 6 [33,39]. It should be emphasized that neither adenosine nor D-ribose 5-monophosphate, which lack either the phosphate group or the stacking moiety and N7, are able to promote the reaction [33]; in fact, due to the interaction of these ligands with the metal ion they inhibit the dephosphorylation process. The fact that the ultimate product of the dephosphorylation reaction of ATP, which proceeds via ADP to AMP, further facilitates the dephosphorylation process in the presence of metal ions has led to the question, whether purine-nucleotides played a role in early evolution in paving the way for a genetically based 'RNA World' [38].



As far as the present discussion is concerned, Fig. 6 offers a further important insight into the reaction mechanism, i.e., tubercidin 5'-monophosphate ($TuMP^{2-}$ = 7-deaza-AMP^{2-}; cf. Fig. 8, *vide infra*), which has the same self-stacking properties as AMP [15] and is structurally completely identical with its parent compound except that N7 is replaced by a CH unit, inhibits the Cu^{2+}-promoted dephosphorylation of ATP. This proves unequivocally that N7 is the crucial binding site for the metal ion already bound to the phosphate group of the other nucleotide, as shown in the structures (cf. Figures 4 and 7).

The further facilitation of the Cu^{2+}-promoted ATP dephosphorylation by AMP is due to the preferred formation of mixed AMP-ATP stacks. These mixed stacks are favored over the formation of pure ATP stacks by statistics (concentration) and by the smaller charge repulsion in $[(AMP)(ATP)]^{6-}$ than in $[(ATP)_2]^{8-}$. With this and the indicated results of Fig. 6 in mind one may propose the structure shown in Fig. 7 for the most reactive mixed AMP-ATP species, i.e., $Cu_3(ATP)(AMP)(OH)^-$, which is derived from the structure seen in Fig. 4 [38]. Of course, the question that needs now to be asked is: Can IMP^{2-} and GMP^{2-} also take over the structuring role of the one ATP in $[Cu_2(ATP)]_2(OH)^-$? The results of Fig. 6 show that this is possible. Though the experiments with IMP and GMP are somewhat hampered due to precipitation, it is evident that the facilitating impact on the Cu^{2+}-promoted ATP dephosphorylation decreases in the series, $AMP^{2-} > GMP^{2-} > IMP^{2-}$, which parallels the decreasing self-stacking tendency of these purine-nucleoside 5'-monophosphates (see Section 2). In other words, these results parallel those summarized in Fig. 5. However, the

Fig. 6. Influence of purine-nucleoside 5'-monophosphates and analogues (given as [NMP] $\times 10^3$) (cf. Fig. 8, *vide infra*) on the initial rate $v_0 = d[P_i]/dt$ (Ms^{-1}) [12] of dephosphorylation of the 1:1 (open symbols; $[Cu^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M) and 2:1 (solid symbols; $[Cu^{2+}]_{tot} = 2 \cdot 10^{-3}$ M and $[ATP]_{tot} = 10^{-3}$ M) Cu^{2+}/ATP systems in aqueous solution at pH_0 6.70 (I = 0.1 M, $NaClO_4$; 50°C). The broken lines indicate uncertainty due to precipitation. This figure combines part of the results shown in Figures 9 and 10 of [33] as well as in Figure 3 (PMEA) of [39].

fact that the antiviral 9-[2-(phosphonomethoxy)ethyl]-adenine (PMEA; see Fig. 8; its metal ion-binding properties were reviewed [40]) facilitates the Cu^{2+}-promoted ATP dephosphorylation even more than its parent nucleotide shows that the formation of the reactive intermediate is a delicate matter. The stacking property as well as the metal ion affinity of the adenine residue in AMP and PMEA are of course identical. Hence, the reason for the larger effectivity of $PMEA^{2-}$ is probably the greater flexibility of its 'aliphatic' chain (see Fig. 8), compared with the more rigid ribosyl moiety of AMP^{2-}, and that this allows a better fit of the various units forming the reactive intermediate.[b]

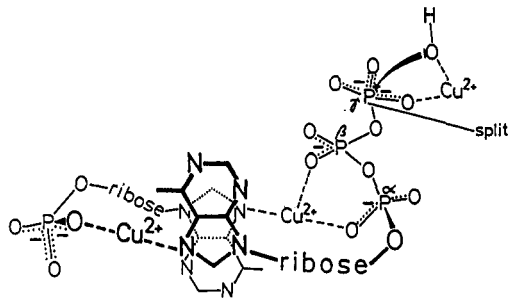


Fig. 7. Probable structure of the reactive $Cu_3(ATP)(AMP)(OH)^-$ species (cf. with Fig. 4). The intramolecular attack of OH^- is indicated on the right-hand side, while the left-hand side shows the metal ion bridge stabilizing the purine stack by M^{2+} coordination to the phosphate group of AMP^{2-} and to N7 of ATP^{4-}.

How sensitive the structure of the reactive intermediate is, also becomes clear from the experiments shown in Fig. 6 with 1,N^6-ethenoadenosine 5'-monophosphate (ε-AMP^{2-}; Fig. 8) and adenosine 5'-monophosphate N(1)-oxide ($AMP{\cdot}NO^{2-}$; Fig. 8). The stacking properties of ε-AMP^{2-} [25,41] are very similar to those of AMP^{2-} and the same may be surmised for $AMP{\cdot}NO^{2-}$. However, both AMP derivatives form stable *chelates* with Cu^{2+} at pH 6.7: In Cu(ε-AMP) the metal ion is bound to the phosphate group and the "1,10-phenanthroline"-like N6,N7 site [25,41] and in $Cu(AMP{\cdot}NO{-}H)^-$ to the ionized *o*-amino *N*-oxide group [42]. This leads to different orientations of the metal ion in space, and consequently, both AMP derivatives are strong inhibitors of the dephosphorylation in the Cu^{2+}/ATP system.

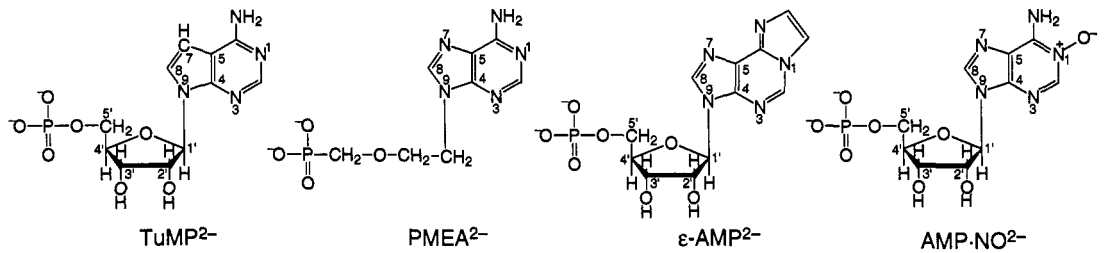


Fig. 8. Chemical structures of AMP^{2-} analogues: tubercidin 5'-monophosphate ($TuMP^{2-}$ = 7-deaza-AMP^{2-}), dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$), 1,N^6-ethenoadenosine 5'-monophosphate (ε-AMP^{2-}), and adenosine 5'-monophosphate N(1)-oxide ($AMP{\cdot}NO^{2-}$).

5. CONCLUSION

The results summarized in this account show how weak interactions, i.e., aromatic-ring stacking, affect the formation of a reactive intermediate and how delicate the structure of this intermediate, in the present case $[M_2(ATP)]_2(OH)^-$, is (Fig. 4). Recent studies with the closely related Cu^{2+}/ADP system show [43] that also in this case the reactive intermediate contains two metal ions per nucleotide and that the initial dephosphorylation rate depends on the square of the reactant concentration. In other words, the most reactive intermediate has the composition $[Cu_2(ADP)]_2(OH)^+$ [43]. Most important in the present context is, however, that again a structure analogous to the one shown in Fig. 4 can be proposed and that the structuring ADP^{3-} in this dimer can also be replaced by a purine-NMP^{2-}; i.e., the facilitating effect on the

b) In this context one should emphasize that rate comparisons need to be done with great care. For example, ATP^{4-} hydrolyzes in a 10^{-3} M solution at pH_0 9.5 (I = 0.1 M, $NaClO_4$; 50°C) with $v_0 = 0.015{\cdot}10^{-8}$ Ms^{-1}, whereas under the same conditions but in the presence of a twofold excess of Cu^{2+} at pH_0 5.5, where the reaction proceeds via $[Cu_2(ATP)]_2(OH)^-$, $v_0 = 30{\cdot}10^{-8}$ Ms^{-1} [12]; i.e., Cu^{2+} promotes the hydrolysis of ATP^{4-} by a factor of 2000. For solutions with [ATP] = 0.1 M the corresponding rates are: ATP alone, $v_0 = 1.5{\cdot}10^{-8}$ Ms^{-1}; with Cu^{2+}/ATP = 2:1, $v_0 = 3{\cdot}10^{-3}$ Ms^{-1}; hence, now the promotion factor equals 200000 [12]. At pH_0 6.70 (Fig. 6) the corresponding promotion factors due to Cu^{2+} are only slightly smaller [12]. The recent conclusion regarding non-enzymatic reactions [7] (cf. also [13]), however, that "there is little effect of coordination by ... divalent metal ions on the rate of ATP hydrolysis" is not valid as the given examples demonstrate. The pitfalls connected with comparisons of the indicated kind have been discussed (see pp. 515-519 in [12]).

Cu^{2+}-promoted ADP dephosphorylation decreases in the series, $AMP^{2-} > GMP^{2-} > IMP^{2-}$ [43], emphasizing again the role of the aromatic-ring stacking in the formation of the reactive intermediate.

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