Facilitation of the copper(II)-promoted dephosphorylation of adenosine 5′-triphosphate (ATP^{4-}) by the antiviral nucleotide analogue, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA)‡

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The antiviral PMEA is able to mimic the structuring adenosine 5′-monophosphate (AMP^{2-}) in the reactive intermediate, $[Cu_3(ATP)(AMP)(OH)]^-$, and to promote the dephosphorylation of ATP; PMEA is about twice as effective as (the ultimate product of this hydrolysis) AMP.

Mechanistic studies on the metal ion-promoted hydrolysis of nucleoside 5′-triphosphates (NTP^{4-}) to nucleoside 5′-diphosphates (NDP^{3-}) and inorganic phosphate (P_i) revealed for this most simple transphosphorylation reaction, which consists of the transfer of a phosphoryl group to water, that the most reactive species contain two metal ions per NTP^{4-}.[1,2] This reflects the known 'general need' for two metal ions in enzymatic phosphoryl transfer reactions.[3] The *in vitro* dephosphorylation of ATP^{4-} in the presence of divalent metal ions (M^{2+}) proceeds *via* dimers of composition $[M_2(ATP)]_2$.[2] Depending on the kind of metal ion involved, hydrolysis occurs either *via* intermolecular H_2O attack or *via* intramolecular hydroxide attack of a phosphate-coordinated $M(OH)^+$ unit,[1b] as is the case with Cu^{2+}. Hence, $[Cu_2(ATP)]_2(OH)^-$ is the most reactive species for Cu^{2+}-promoted dephosphorylation.[1b,2] Such studies with copper are of general interest due to the evidence that there is an ATP-dependent Cu^{II} transporter in the Golgi apparatus[4] and the observation that the Menkes P-type ATPase is a transmembrane copper-translocating pump, which is defective in the human disorder called Menkes disease.[5] There is also a somewhat older suspicion that $Cu(ATP)^{2-}$ is involved in the inhibition of human erythrocyte ($Ca^{2+} + Mg^{2+}$)-ATPase.[6] Mixed Cu(ATP)(L) complexes (L = tryptophanate, *etc*. see also below) protect ATP against hydrolysis.[2]

In the reactive dimeric $[M_2(ATP)]_2$ complexes, which occur in low concentration and involve purine stacking[7] and N^7-M^{2+} coordination, one of the two ATPs takes over a structuring role and thus acts as its own hydrolytic 'enzyme'.§ This role cannot be taken over by adenosine, D-ribose 5-monophosphate or by tubercidin 5′-monophosphate ($TuMP^{2-}$, 7-deaza-AMP^{2-}) because these ligands are unable to form the required bridge in the reactive species, but it can be taken over by adenosine 5′-monophosphate (AMP^{2-}). In the presence of Cu^{2+}, this then leads, by promoting further the reactivity of the Cu^{2+}/ATP system, to the reactive $[Cu_3(ATP)(AMP)(OH)]^-$ species shown in Fig. 1.[8a] How does the related antiviral PMEA behave in this reaction? This adenine-nucleotide analogue (see Fig. 2) is active against various viruses, including hepatitis B (HBV) and human immunodeficiency viruses (HIV-1 and HIV-2).[9,10] After its twofold phosphorylation by cellular nucleotide kinases,[11] the resulting triphosphate analogue can serve as a substrate for the viral DNA polymerase or reverse transcriptase and subsequently terminates the growing nucleic acid chain.[12] Thus it was interesting to see whether $PMEA^{2-}$ would be able to mimic AMP^{2-} in the reactive intermediate (Fig. 1) and to facilitate thus also the Cu^{2+}-promoted dephosphorylation of ATP^{4-}.

The results of our experiments¶ are shown in Fig. 3 in parallel with the data obtained with AMP under identical conditions. These latter data agree (within the error limits) with those obtained previously.[1b] The addition of an approximately sevenfold excess of AMP to the Cu^{2+}/ATP 2:1 system facilitates the dephosphorylation reaction by a factor of about three (Fig. 3).‖ Indeed, $PMEA^{2-}$ can take over the structuring role of AMP^{2-} (or ATP^{4-}) in the reactive species (Fig. 1) and, astonishingly, it is twice as effective!** This is possibly due to the somewhat increased flexibility of $PMEA^{2-}$ compared with AMP^{2-} as a result of the replacement of the ribose residue by the open chain (see Fig. 2).†† To our knowledge, this is the first example demonstrating in a 'simple' reaction a close relationship between PMEA and its parent nucleotide AMP.

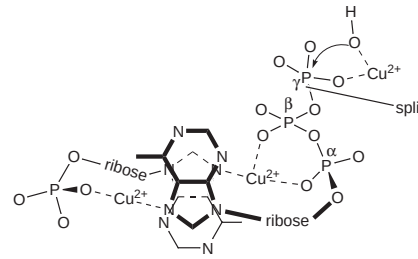

Fig. 1 Probable structure of the reactive $[Cu_3(ATP)(AMP)(OH)]^-$ species. The intramolecular attack of OH^- is indicated on the right-hand side, while the left-hand side shows the metal ion bridging which stabilizes the purine stack by coordination to the phosphate group of AMP^{2-} and to N^7 of ATP^{4-}. In the reactive $[M_2(ATP)]_2(OH)^-$ dimer the structuring ATP^{4-} occupies formally the left side in the above structure.

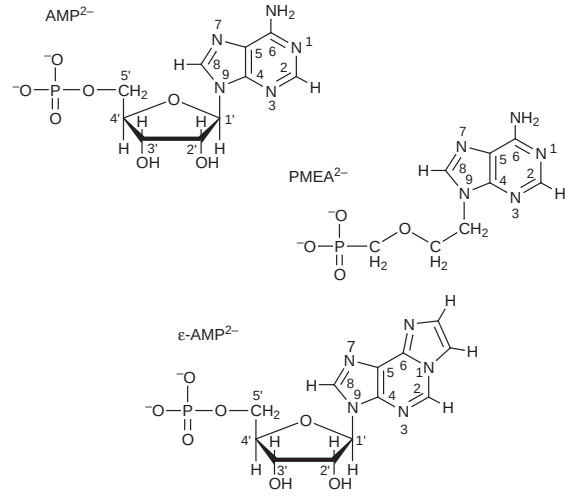

Fig. 2 Structure of the dianion of 9-[2-(phosphonomethoxy)-ethyl]adenine ($PMEA^{2-}$) in comparison with those of adenosine 5′-monophosphate (AMP^{2-}) and 1,N^6-ethenoadenosine 5′-monophosphate (ε-AMP^{2-})

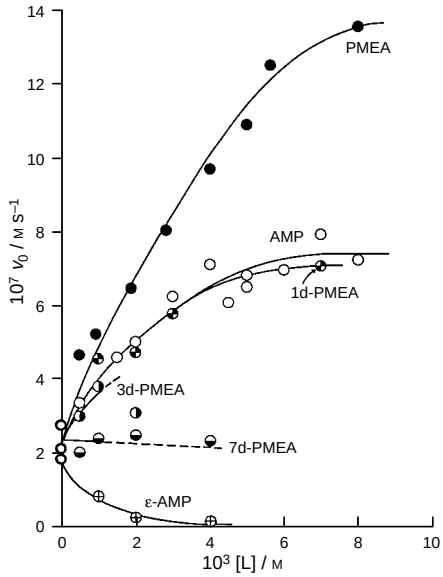

Fig. 3 Influence of PMEA (●) and AMP (○) as well as of some related derivatives (L) on the initial rate v_o (м s^{-1}) of dephosphorylation of the Cu^{2+}/ATP 2 : 1 system ($[Cu^{2+}]_{tot} = 2 \times 10^{-3}$ м and $[ATP]_{tot} = 10^{-3}$ м) in aqueous solution at pH_o 6.70 (I = 0.1 м, $NaClO_4$; 50 °C).¶ The broken lines indicate uncertainty due to precipitation.

These observations initiated further experiments with the deaza derivatives of PMEA, in which the three aromatic nitrogens, N^1, N^3, and N^7 (see Fig. 2), are systematically replaced by a CH unit. Fig. 3 shows that 1-deaza-$PMEA^{2-}$ facilitates the Cu^{2+}-promoted ATP^{4-} dephosphorylation at pH 6.7 about as well as AMP^{2-}.‡‡ Unfortunately, addition of 3-deaza-PMEA and 7-deaza-PMEA to a Cu^{2+}/ATP 2 : 1 system leads, even at relatively low concentrations, to turbidity of the reaction solution and finally to a precipitate. Nevertheless it is evident that 3-deaza-$PMEA^{2-}$ can also facilitate the reaction while some inhibition of the system occurs on addition of 7-deaza-$PMEA^{2-}$. This agrees with previous observations made with $TuMP^{2-}$ (7-deaza-AMP^{2-})[1b] which had confirmed the earlier suggestion[1a] regarding the importance of N^7 for the structure of the reactive intermediate.[2,8a]

To demonstrate how delicate the structure of the reactive intermediate (Fig. 1) is, we repeated a previous Cu^{2+}/ATP 1 : 1 experiment[1b] with 1,N^6-ethenoadenosine 5′-monophosphate (ε-AMP^{2-}; Fig. 2) under the present Cu^{2+}/ATP 2 : 1 conditions. The inhibition of the reaction is dramatic (Fig. 3); a fourfold excess of ε-AMP prevents the dephosphorylation of ATP^{4-} almost completely. The stacking properties of ε-AMP^{2-} are very similar to those of AMP^{2-};[13] however, in Cu(ε-AMP) the metal ion is bound to the phosphate group and the '1,10-phenanthroline'-like N^6, N^7 site[13] which leads to a different orientation of the metal ion in space. Consequently, this AMP derivative cannot take over the structuring role needed in the reactive species (Fig. 1), but it strongly inhibits, as is usual for ternary Cu(ATP)(L) complexes,[1b] the dephosphorylation reaction of the Cu^{2+}/ATP system. This inhibition re-emphasizes how well $PMEA^{2-}$ is suited to mimic AMP^{2-} in certain reactions.

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Notes and References

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‡ This is part 12 of 'Hydrolysis of Nucleoside Phosphates'; for part 11 see ref. 14.

§ In the presence of metal ions these adenine-nucleotide systems[8a] show thus related properties to ribozymes;[8b] their possible role in early evolution has been discussed.[8a]

¶ The disodium salt of ATP (> 98%; p.a.) was obtained from Serva Feinbiochemica GmbH, Heidelberg, Germany. The other reagents were the same as used previously.[15] The liberated P_i was determined by the molybdate method;[1,14] a critical summary of the methodology is given in ref. 2. No buffers were used to adjust the pH of the solutions because they inhibit metal ion-promoted dephosphorylation reactions.[2] Instead, the pH was adjusted with conc. NaOH or $HClO_4$ using a glass rod ('dotting'; volume changes negligible).[1,2] To obtain the initial pH, *i.e.* pH_o = 6.70, and the corresponding initial rate for the dephosphorylation, $v_o = d[P_i]/dt$ (м s^{-1}), at least two experiments were carried out in this pH range; these results were then interpolated to the desired pH_o as shown, *e.g.* in Figs. 1 and 3 of ref. 1*a* and 2, respectively. Usually v_o is reproducible to ±10%, but for a very rapid reaction and/or an unstable pH the error limit may increase to ±25%. Attempts to carry out the analogous experiments with Zn^{2+} failed due to precipitation; the very low solubility of Zn(PMEA) is known.[16a]

‖ ATP^{4-} hydrolyzes in a 10^{-3} м solution at pH_o 9.5 (I = 0.1 м, $NaClO_4$; 50 °C) with $v_o = 0.015 \times 10^{-8}$ м s^{-1}, whereas in a solution of the same ATP concentration and under the same conditions but in the presence of a twofold excess of Cu^{2+} at pH_o 5.5, where the reaction proceeds *via* $[Cu_2(ATP)]_2(OH)^-$, $v_o = 30 \times 10^{-8}$ м s^{-1};[2] *i.e.* Cu^{2+} promotes the hydrolysis of ATP^{4-} by a moderate factor of 2000. For solutions with [ATP] = 0.1 м the corresponding rates are: ATP alone, $v_o = 1.5 \times 10^{-8}$ м s^{-1}; with Cu^{2+}/ATP = 2 : 1 $v_o = 3 \times 10^{-3}$ м s^{-1}; hence, one obtains a large promotion factor of 2×10^5.[2] At pH_o 6.70 (Fig. 3) the corresponding promotion factors due to Cu^{2+} are only slightly smaller.[2] The pitfalls connected with comparisons of the indicated kind have been discussed in ref. 2 (pp. 515–519). Therefore, conclusions regarding nonenzymatic reactions (*e.g.* ref. 3,17), that divalent metal ions have little effect on the rate of ATP hydrolysis, have to be considered with care; much depends on the metal ion and the conditions (pH, *etc.*) involved.

** This is despite the higher basicity of the phosphonate group in $PMEA^{2-}$ (pK_a = 6.90)[15] compared to that of the phosphate group in AMP^{2-} (pK_a = 6.21)[18] which disfavours Cu^{2+} binding of $PMEA^{2-}$ (due to the competition with H^+) by a factor of about 0.5.

†† For the M^{2+}-binding properties of PMEA see ref. 16.

‡‡ Hence, N^1 has no significant role in the Cu^{2+}-promoted hydrolysis of ATP which contrasts with a recent claim[19] made for the Zn^{2+}/ATP system.

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