Have adenosine 5′-triphosphate (ATP^{4-}) and related purine-nucleotides played a role in early evolution? ATP, its own 'enzyme' in metal ion facilitated hydrolysis!*

Helmut Sigel

Institute of Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel (Switzerland)

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Abstract

Had ATP a directing role in early evolution? Mechanistic studies on the *in vitro* dephosphorylation of adenosine 5′-triphosphate (ATP^{4-}) revealed that in the presence of metal ions (M^{2+}), like Zn^{2+}, Cd^{2+} or Cu^{2+} the reaction proceeds via dimeric species, i.e. preferably via $[M_2(ATP)]_2(OH)^-$ or $[M_2(ATP)]_2$ depending on pH. These dimers occur in low concentration and involve purine stacking and a M^{2+}/N-7 interaction. In other triphosphate monoesters with a non-coordinating organic residue including pyrimidine-nucleoside 5′-triphosphates, which are considerably less reactive than the ATP systems, monomeric species govern the reactivity. The reason for the high reactivity of the M^{2+}/ATP dimers is that one of the two ATPs takes over a structuring role and thus ATP acts as its own hydrolytic 'enzyme'. It is noticeable that this structuring role can also be adopted by purine-nucleoside 5′-monophosphates, AMP^{2-} being especially effective. Based on these observations it is suggested that ATP may have played a directing role in early evolution, i.e. in a primitive metabolism without genetics. To say it differently, due to its self-activating properties ATP might have been able to stick out of the chemical 'noise' present on the early earth. In this way ATP might have participated in paving the way for a genetically based 'RNA World'. The role of Mg^{2+} and other metal ions as well as that of adenosine 5′-diphosphate (ADP) and of other purine-nucleotides is also discussed.

Introduction

At the very latest since Szent-Györgyi [1] postulated about 35 years ago a macrochelated structure for the Mg^{2+} complex of ATP**, this nucleotide and its various complexes have fascinated coordination chemists and biochemists alike. Studies have been hampered for many years due to the unrecognized self-association of ATP [2, 3], but significant progress has been achieved over the past 10 years [2, 4] and the metal ion-coordinating properties of ATP in diluted aqueous solution are now relatively well understood [5, 6]; though this conclusion does not mean that all relevant questions have been solved [5].

In this essay I would like to focus on the *reactive* properties of ATP and as this essay is written for the 200th jubilee issue of *Inorganica Chimica Acta* it appears appropriate to indicate unsolved problems as a stimulant for further research. This aim is tackled by rising questions and by presenting in part also rather speculative answers. Much of the present knowledge on the metal ion facilitated dephosphorylation of ATP has recently been reviewed and summarized in detail [7]. Therefore, in various following sections only results and conclusions pertinent to the present topic will be indicated without giving the detailed experimental proofs, which are easily accessible in the literature (see ref. 7 and the references cited therein).

Evolution and the 'RNA World'

The origin of life has always fascinated mankind and many scientists have already struggled with this and

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**Abbreviations: Ado, adenosine; ADP^{3-}, adenosine 5′-diphosphate; AMP^{2-}, adenosine 5′-monophosphate; ATP^{4-}, adenosine 5′-triphosphate; ϵ-ATP^{4-}, 1,N^6-ethenoadenosine 5′-triphosphate; bpy, 2,2′-bipyridyl; CTP^{4-}, cytidine 5′-triphosphate; dien, diethylenetriamine = 1,4,7-triazaheptane; GTP^{4-}, guanosine 5′-triphosphate; ITP^{4-}, inosine 5′-triphosphate; M^{2+}, divalent metal ion; NTP^{4-}, nucleoside 5′-triphosphate; PO_4, if nothing else is specified, the formula PO_4 represents all related species which might be present in solution, i.e. H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}; $RibMP^{2-}$, D-ribose 5′-monophosphate; TTP^{4-}, thymidine 5′-triphosphate; UTP^{4-}, uridine 5′-triphosphate. For further abbreviations see Figs. 1 and 7.

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related questions (e.g. refs. 8–14). The present conventional wisdom implies that some 4 billion years ago life started out in an 'RNA World' [15], a term coined by Gilbert in 1986 [16]. This hypothesis found widespread acceptance in the early 1980s and is based on the discovery of Cech and Altman that RNA is able to catalyze reactions in much the same way as proteins [15, 16]. Such proteins with catalytic properties are known as *enzymes* and correspondingly RNAs catalyzing reactions became now known as *ribozymes*. Indeed, catalytic RNA segments have proved to be quite versatile in snipping themselves, moving the pieces around and splicing themselves together again, or in incorporating other RNA molecules [15–17].

As RNA is also part of the cell's genetic machinery the RNA-World scenario explains many things well and it eliminates the chicken-egg paradox [15–17] one is faced with if proteins are employed as enzymes and DNA as the carrier of the genetic information; because: which came first, DNA or proteins? The problem is, proteins cannot form without DNA, and DNA not without proteins. It is evident that the RNA-World idea is appealing because both DNA and proteins are descendents of RNA. In the view of Gilbert (see ref. 16), and in line with earlier suggestions of Crick, Orgel and Woese [15], the first organisms consisted of RNA. As these organisms evolved they learned to synthesize proteins, which could help them to replicate more efficiently, and finally the RNA organisms gave rise to DNA; a molecule more reliable in storing the genetic information.

However, if life began with RNA then RNA itself must have formed by spontaneous chemical reactions among simpler molecules. This is quite a demanding condition and Orgel concludes that some simpler molecule may well have paved the way for RNA [16]. The question is, what?

Indeed, many origin of life researchers are now convinced that some crucial concept is still missing [15, 16]. For example, de Duve [18] goes back to an older idea that life originated with a kind of primitive metabolism. He concludes [15] that eventually a network of interrelated catalysts and reaction products, thioesters playing a critical role [16, 18], began to rise above the chemical 'noise', thus providing a form of natural selection without genetics.

Possible primordial advent of AMP, ADP and ATP

It is my belief that AMP, ADP and ATP are low-molecular-weight molecules well suited to participate in an early primitive metabolism of the type indicated above. AMP and ATP, as shown below, and most probably also ADP appear to be able to promote certain reactions, *in combination with metal ions*, beyond the chemical 'noise' originally present on the early earth; in this way they would give rise to selectivity and a kind of primitive metabolism. As will be discussed further below, ATP in the form of $[M_2(ATP)]_2(OH)^-$ is a highly reactive species for the reaction:

$$ATP + H_2O \longrightarrow ADP + PO_4 \qquad (1)$$

One of the two ATPs in the mentioned dimer takes over a structuring role and thus ATP acts as its *own* hydrolytic 'enzyme' [19, 20].

That AMP, and consequently also ADP and ATP were formed at a very early stage is quite plausible. HCN is considered as one of the common starting materials available on the early earth for prebiotic syntheses [9, 12, 21, 22]. It oligomerizes to produce a complex range of products among them adenine [22–25] and, under somewhat different conditions also traces of uracyl [22, 26], a further important nucleic base residue (cf. also ref. 25). Formaldehyde, photochemically easily formed from CO_2 and water vapour [22, 27], is another apparently available and important starting material for prebiotic syntheses [9]; it has been shown to accelerate the mentioned oligomerization [22, 28].

In the presence of alkaline catalysts, formaldehyde is able to produce a whole range of sugars, including β-D-ribose in the so-called *formose reaction* [25, 29, 30]. There are also proposals how a base and a sugar could be fused to a nucleoside [25]; e.g. using the salts obtained by evaporating sea water and heating the whole mixture yields nucleosides, and adenosine has been produced in this way.

Phosphorylation of nucleosides has been achieved by heating them in salt mixtures in various ways [31]. For example, a system containing urea and hydroxyapatite is rather effective, and it is generally believed that hydroxyapatite was common on the primitive earth since it is the most abundant source of phosphate on earth today. Urea is also formed in many prebiotic reaction sequences [31].

Finally, Mg^{2+} is known [31] to have a specific catalytic effect in the presence of urea and inorganic phosphate, for it strongly enhances the formation of pyrophosphate bonds under conditions expected on the primitive earth. Hence, by this mechanism ADP and ATP could have formed from AMP. The role of Mg^{2+} in this event is especially notable, as this metal ion is a poor catalyst for the dephosphorylation reaction of ATP in aqueous solution ([7], see also below). One may further add here that in dilute aqueous solution at neutral pH [32] also Mg^{2+} is a promoter for the generation of ATP from acetyl phosphate and ADP in the presence of the (24)-N_6O_2 macrocycle.

To conclude, phosphorylation of nucleosides and the formation of pyrophosphate bonds may have occurred on the early earth by purely thermal processes [31]. The resulting compounds could then have been important starting materials for further syntheses in aqueous solution [31] or on surfaces [33]. Here ATP may well have played a significant role (see below) as it appears to be able to participate in, what Eigen calls [16], directed evolution. It is evident that formation of a nucleotide, like AMP, from the above mentioned precursor molecules is a much simpler task than the formation of RNA, where a legion of similar polymers could form [15] by combination of its precursor molecules. Yet once ATP is formed, from AMP, it might well have paved the way, as discussed below, for the formation of oligonucleotides and finally RNA.

Why is ATP special?

The mechanistic aspects of the metal ion promoted hydrolysis of nucleoside 5′-triphosphates have recently been reviewed and summarized [7]. In the present context I would only like to point out that from the six natural NTPs depictured in Fig. 1 ATP represents a special case.

In Fig. 2 is plotted the (pseudo) first-order rate constant for the dephosphorylation of NTPs in dependence on pH [34, 35]. It is evident that independent of the nucleic base residue all six NTPs show the same pH-rate profile. Individual properties of the NTPs only become apparent in the presence of one equivalent of Cu^{2+}; though even under these conditions the pyrimidines CTP, UTP and TTP show the same properties up to pH 8, the differences at pH > 8 being due to the deprotonation of the H(N-3) site [7]. That it is indeed the base residue that affects the different reactivities of the NTPs is demonstrated by the addition of one equivalent of 2,2′-bipyridyl to the Cu^{2+}–NTP 1:1 systems: under the experimental conditions mixed ligand $M(bpy)(NTP)^{2-}$ complexes are formed and in these species any metal ion nucleic base interaction is prevented [6, 36, 37]. Consequently, the pH-rate profile for all 6 systems is again identical [7]. In addition it may be pointed out, as is evident from Fig. 2, that the NTPs are more stable toward hydrolysis in the mixed complexes than in the free, uncomplexed NTPs. This observation is meaningful with regard to today's biological systems as it allows transport of the sensitive NTPs, e.g. through membranes, in the form of mixed

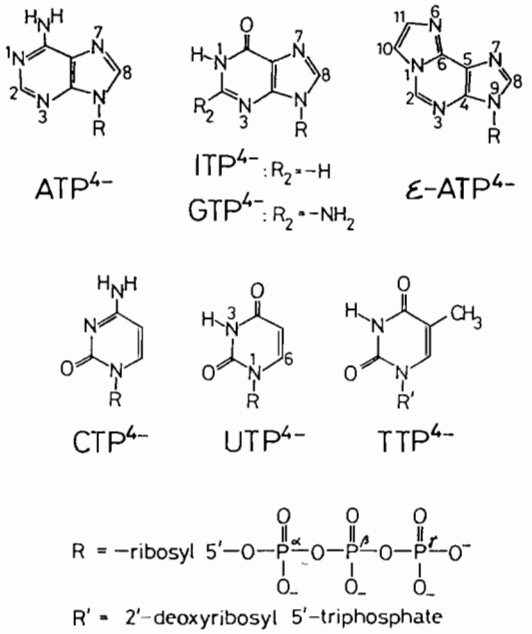

Fig. 1. Chemical structures of several nucleoside 5′-triphosphates (NTP^{4-}), together with the labeling system for the triphosphate chain; note, the phosphate groups in the NTPs are labeled α, β and γ, where γ refers to the terminal phosphate group. Obviously, the analogous nucleoside 5′-monophosphates (NMP^{2-}) or nucleoside 5′-diphosphates (NDP^{3-}) have the corresponding structures with one or two phosphate groups, respectively.

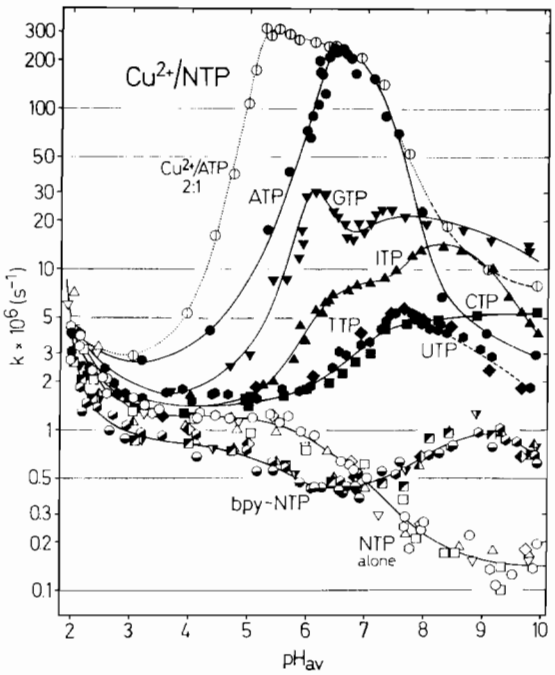

Fig. 2. Comparison of the Cu^{2+}-promoted dephosphorylation of ATP (●), ITP (▲), GTP (▼), CTP (■), UTP (●), and TTP (◆) (always in the ratio 1:1) in aqueous solution as a function of pH, characterized as the first-order rate constants k (s^{-1}) [7]. For comparison is shown also the rate for the Cu^{2+}/ATP 2:1 system (⊙; dotted line). In addition is given ATP (○), ITP (△), GTP (▽), CTP (□), UTP (◇) and TTP (◇) alone, and ATP (◒), ITP (◭) GTP (◬), CTP (◩), UTP (◪) and TTP (◈) in the presence of Cu^{2+} and 2,2′-bipyridyl (1:1:1). The concentration of each NTP was always 10^{-3} M; I = 0.1 M, $NaClO_4$; 50 °C. The broken line portions indicate uncertainty due to precipitation. The above figure combines results from Figs. 2 and 13 of ref. 35 and Fig. 1 of ref. 38 (see also ref. 34).

ligand complexes; similarly, on the early earth, once formed, these 'energy-rich' compounds could be 'stored' via mixed ligand species.

The results of comprehensive mechanistic studies [7] are summarized in Fig. 3 [34, 38, 39]. The most reactive species for the dephosphorylation of the pyrimidine nucleoside 5'-triphosphates has the composition $M_2(NTP)(OH)^-$ [$=M_2(R\text{-}TP)(OH^-)$]. In these cases the base moiety is not participating in the reaction, i.e. these NTPs behave just as methyl triphosphate. A tentative structure of the reactive species is shown in A of Fig. 3. It should be emphasized that in a $M(NTP)^{2-}$ species the metal ion is (α,) β and γ phosphate coordinated. Addition of a second metal ion produces then in equilibrium a species where one metal ion is forced into an α,β coordination and the other, carrying the nucleophile OH^-, is bound to the terminal γ phosphate group.

That macrochelate formation is important in the metal ion promoted hydrolysis of ATP goes back to an early suggestion of Szent-Györgyi [1]. However, such a reactive species has never been observed in ATP systems; only very recently it was discovered in the Cu^{2+} system of ϵ-ATP [39, 40], a nucleotide which does not occur in nature (for the structure see Fig. 1). It is evident from B in Fig. 3 that the nucleic base backbinding of one of the two metal ions facilitates its coordination to the β,γ phosphate groups. Otherwise the mechanistic details and the composition of the most reactive species are the same for A and B in Fig. 3.

It is only with ATP that a dimer with a M^{2+}:ATP ratio of 2:1 is formed ($M^{2+}=Ni^{2+}$, Zn^{2+} or Cd^{2+}, and

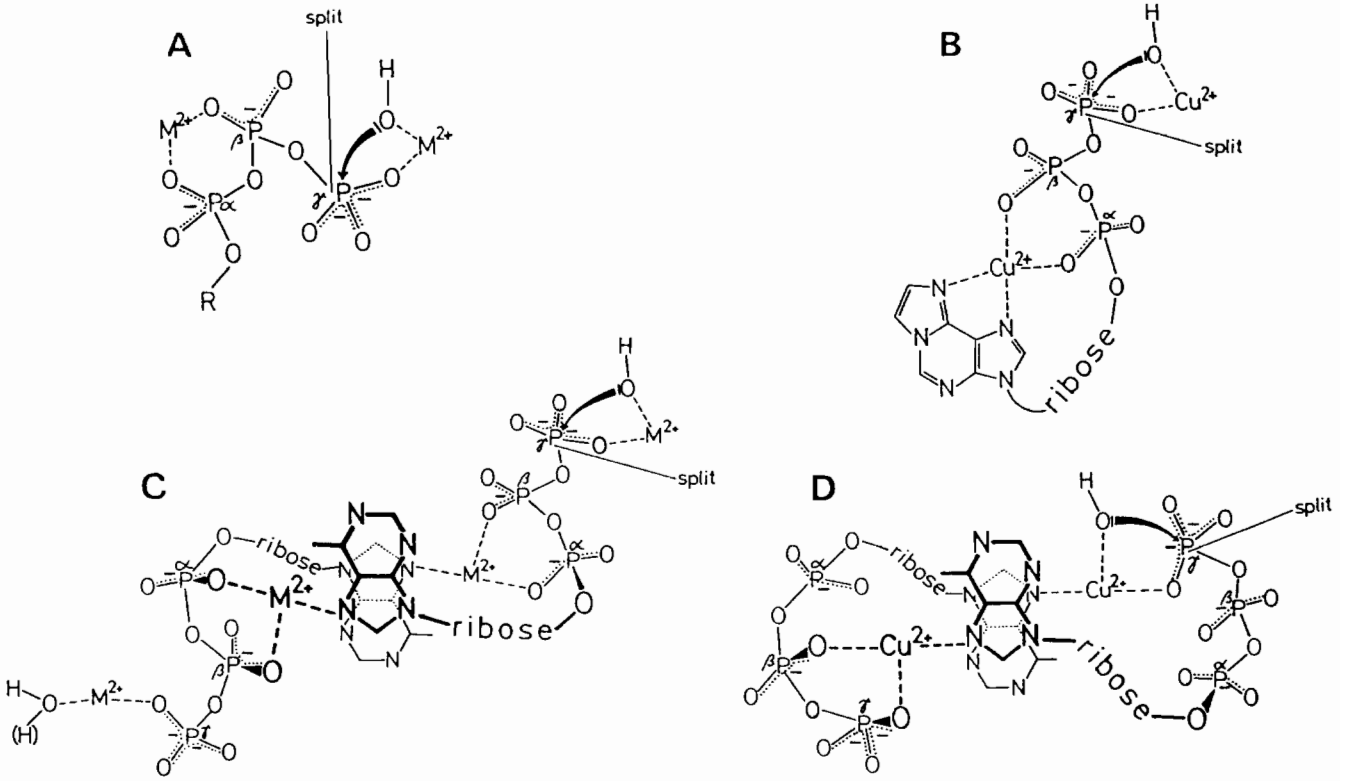

Fig. 3. Comparison of the three types of most reactive species which contain a metal ion-to-nucleotide ratio of 2:1 and which are formed during the metal ion promoted dephosphorylations of nucleoside 5'-triphosphates. A: probable structure of the reactive $M_2(R\text{-}TP)(OH)^-$ species formed with triphosphates having a non-coordinating organic residue R [38]. B: example of a monomeric reactive species with metal ion backbinding to well-positioned sites of the organic residue; the probable structure of the reactive $Cu_2(\epsilon\text{-}ATP)(OH)^-$ complex is shown [39]. C: proposed structure for the reactive $[M_2(ATP)]_2(OH)^-$ dimer, which occurs in low concentration during the dephosphorylation of ATP and other purine-NTPs [34]. 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). For reasons of completeness is also given, D: tentative structure of the reactive $[Cu(ATP)]_2(OH)^{5-}$ dimer, which occurs in low concentration during the Cu^{2+}-promoted dephosphorylation of ATP at pH $\geqslant$6.5 [34] (see the comment in the footnote on p. 5). The intramolecular attack of OH^- is indicated on the right-hand side, while the left-hand side shows a Cu^{2+} ion stabilizing the dimer by coordination to the γ,β-phosphate groups of one ATP and to N-7 of the other.

Cu^{2+} at pH < 6.5)*; the tentative structure of the most reactive species $[M_2(ATP)]_2(OH)^-$ is shown in C of Fig. 3. There are two points which should be emphasized: (i) One ATP serves to bring the other into the reactive state. Note, stacking and M^{2+}-(N-7) interactions are important features of this structure. (ii) Like in the structures A and B also two metal ions are needed at a given triphosphate residue. As only one of the two M^{2+} ions bound to the triphosphate chain is also interacting with N-7 of the neighboring ATP it is evident that the two metal ions have different roles and one may ask if synergistic effects can be observed in mixed metal ion systems. We shall now address the latter point first.

Why did nature select in many instances Mg^{2+} as the most appropriate metal for ATP?

Figure 4 shows the influence of various metal ions on the rate of ATP dephosphorylation [7, 34] in a Cu^{2+}:ATP 1:1 system at pH 5.5. It is evident that addition of a further equivalent of Cu^{2+} promotes the reaction most significantly. The reason for this observation is that the second Cu^{2+} carries at this pH already an OH^- ion which is ready for an *intra*molecular attack. Zn^{2+} ions carry under these conditions only to a limited extent an OH^-, therefore the additional coordination of Zn^{2+} promotes the reaction less than the additional coordination of Cu^{2+}.

*It should be noted here that the Cu^{2+}/ATP 1:1 system at pH > 6.5 is apparently an exceptional case [7]: Job's series showed [35] that from pH > 6.7 the Cu^{2+}/ATP ratio is 1:1 in the reactive dimer. Indeed, at pH > 6.5 the 2:1 and 1:1 systems share the same reactivity (see Fig. 2) [34, 35]. This indicates that the second Cu^{2+} simply hydrolyzes away in this pH range and is no longer available for promotion of the dephosphorylation; hence $[Cu(ATP)]_2(OH)^{5-}$ is the reactive species. It is also assumed for this complex that both Cu^{2+} bridge an ATP dimer. The reaction is then initiated by a partial release of the $(\alpha),\beta$ group from the coordination sphere of Cu^{2+} upon deprotonation of a coordinated water molecule. This latter Cu^{2+} would then be coordinated in the reactive intermediate to N-7 of one ATP and the γ-phosphate group of the other ATP, allowing intramolecular attack of a coordinated OH^- as shown in D of Fig. 3. It is evident that with progressing hydroxo complex formation the bridging to N-7 and the stability of the dimer will be affected [34], leading to $Cu(ATP)(OH)^{3-}$ (or derivatives thereof [41]) and a decreasing reactivity (see also Fig. 2). However, one must also mention that there is a high probability that the reactive $[Cu(ATP)]_2(OH)^{5-}$ species (Fig. 3D) may only apparently be an exceptional case because the experiments were carried out at $I = 0.1$ M ($NaClO_4$), i.e. Na^+ could well play under these conditions at pH > 6.5 the role of the second metal ion and actively participate in the reaction (*vide infra*, Table 1 and ref. 42); then the structure of the actual reactive species would become similar to the one shown in Fig. 3C.

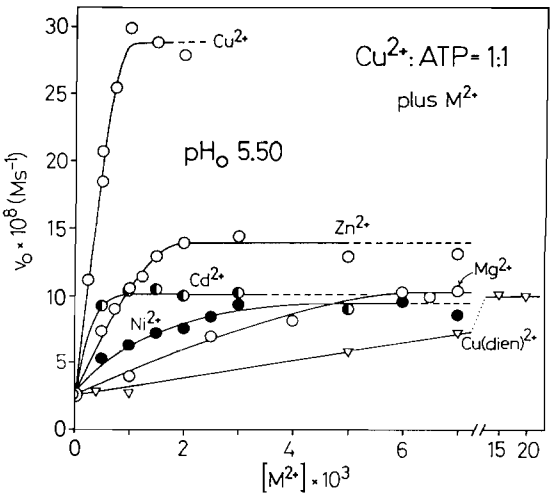

Fig. 4. Dependence of the initial rate, $v_0 = d[PO_4]/dt$ (M s^{-1}), of the Cu^{2+}-promoted dephosphorylation of ATP ($[Cu^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M) in aqueous solution on the addition of further divalent metal ions (○, ◐, ●) or the Cu^{2+} 1:1 complex with diethylenetriamine (dien, ▽) at pH_0 5.50; $I = 0.1$ M, $NaClO_4$; 50 °C. The broken line portions indicate uncertainty due to precipitation. The above figure is constructed with part of the results shown in Fig. 6 of ref. 34.

However, most interesting is the result observed with the ions Ni^{2+}, Cd^{2+} and Mg^{2+}. In these cases no hydroxo complexes can be formed at pH 5.5 and indeed with all three metal ions the same limiting rate is reached. This shows further that in these cases dephosphorylation occurs via an *inter*molecular water attack. This conclusion is confirmed by Cu(dien)$^{2+}$, a species which has only a single position left in the equatorial coordination sphere of Cu^{2+}.

It should be emphasized that Mg^{2+} alone does practically not promote the dephosphorylation of ATP. This observation is most fascinating with regard to evolution, as it has been suggested [31] that Mg^{2+} did play a significant role in the *formation* of pyrophosphate bonds; it would be counter-productive if the same ion would also effectively facilitate its *decomposition*. Instead, Mg^{2+} may be considered as a protector of ATP by its coordination solely to the $(\alpha,)\beta$ and γ phosphate groups. The reason is that Mg^{2+} has only a very low affinity toward N donors. Clearly, such an affinity is crucial for the formation of the most reactive species involving N-7 as shown in C of Fig. 3. Metal ions like Zn^{2+} or Cd^{2+} with a pronounced affinity toward N donors are therefore good promoters.

However, nature has found a way that the apparently unreactive Mg^{2+}/ATP interaction is transformed into a reactive state [7, 34, 42]. One of the important results of Fig. 4 is the observation that Mg^{2+} can further promote the reactivity of $Cu(ATP)^{2-}$! The dimeric structure shown in Fig. 3 makes clear that Mg^{2+} is well suited for the role of this metal ion which has to

coordinate only at the phosphate group and not simultaneously at N-7.

Synergistic effects may not only be observed in the combination Cu^{2+}/Mg^{2+} but also in combinations with other metal ions [7, 42] having a pronounced affinity toward N donors as is evident from the results listed in Table 1. Addition of one extra equivalent of Mg^{2+} at pH 7.5 to a Zn^{2+}:ATP 1:1 system promotes the dephosphorylation reaction by a factor of 2.5. Addition of five equivalents nearly doubles the rate further [42]. Moreover, addition of a 500-fold excess of Na^+ to the Zn^{2+}:ATP 1:1 system also promotes the reactivity; the effect corresponds approximately to that achieved with one extra equivalent of Mg^{2+} (see Table 1).

At this point it should be added that several enzymes catalyzing transphosphorylations contain an intrinsic Zn^{2+}, an extremely versatile metal ion [43–46]; among these enzymes are, for example RNA polymerases [47], which use nucleoside 5′-triphosphates as substrates, but for activity the presence of extrinsic Mg^{2+} (or Mn^{2+}) is also required [48, 49]. Furthermore, from Fig. 3 it is clear that a metal ion coordination of the kind shown will lead to a breakage between the β and γ groups. It is evident that an enforced placement by the enzyme of one metal ion to an α coordination and of the second metal ion to a β,γ coordination will facilitate breakage of the α,β bond, i.e. a nucleoside 5′-phosphoryl or a pyrophosphoryl transfer will result. It is further apparent that one of the two metal ions coordinated to the triphosphate chain may be replaced by a monovalent ion, e.g. Na^+ (see Table 1), or even by an interaction with a positively charged amino acid side chain [7, 42].

TABLE 1. Evidence for synergistic effects in aqueous solution: Promotion of the initial rate of dephosphorylation, $v_0 = d[PO_4]/dt$ ($M\ s^{-1}$) [7], of M^{2+}/ATP 1:1 systems ($[ATP] = [M^{2+}] = 10^{-3}$ M) by the addition of 1 extra equivalent of M^{2+} at pH_0 7.50 ($I = 0.1$ M, $NaClO_4$; 50 °C). The effect of 0.5 M Na^+ is also given for comparison, as are the relative (*RPF*) and the absolute promotion factors (*APF*)[a]

M^{2+}/ATP = 1:1	Extra M^{2+}	$v_0 \times 10^8$	*RPF*[a]	*APF*[a]
Mg^{2+}/ATP	none	0.045	1.0	3.0
	Mg^{2+}	0.050	1.1	3.3
	Zn^{2+}	0.62	14	40
Zn^{2+}/ATP	none	0.25	1.0	17
	Mg^{2+}	0.62	2.5	40
Mg^{2+}/ATP	Na^+(cf.[b])	0.055	1.2	3.7
Zn^{2+}/ATP	Na^+(cf.[b])	0.60	2.4	40

[a]Abstracted from Tables 2 and 3 of ref. 42. The relative promotion factors (*RPF*) are based on the dephosphorylation rate of the corresponding M^{2+}/ATP = 1:1 system, e.g. $RPF = 0.050/0.045 = 1.1$, while the absolute promotion factors (*APF*) are based on the initial dephosphorylation rate of ATP^{4-}, i.e. on $v_0 = 0.015 \times 10^{-8}$ $M\ s^{-1}$ ($I = 0.1$ M, $NaClO_4$; 50 °C); e.g. $APF = 0.045/0.015 = 3.0$. [b]In this special case $[Na^+] = 0.5$ M, while generally Na^+ was used to adjust I to 0.1 M. Note, the increased concentration of Na^+ has a remarkable effect only on the Zn^{2+}/ATP 1:1 system.

I have commented before [7] on the conclusions that may be drawn from the *in vitro* dephosphorylations with regard to enzymic systems. As indicated above the correct positioning of metal ions (or the positive charges) along the phosphate chain is the crucial event. One may imagine that a reorganization of the metal ion binding occurs when, for example, $Mg(ATP)^{2-}$ moves into the active site of an enzyme [7]. Of course, another way one could envisage is a cyclic type of movement in a multi-enzyme complex which then periodically gives rise to the exact positioning needed for an activation and hence transfer of a phosphoryl or nucleotidyl group.

Further considerations on the role of the structuring ATP

If the conclusions presented in connection with C of Fig. 3 and the structure of the dimeric $[M_2(ATP)]_2$ species are correct then one may ask, whether this ATP, which has the structuring role in the dimer cannot be replaced by another ligand. The simplest case of such a ligand which still can stack and which also offers N-7 and a phosphate group is AMP^{2-}. Indeed, addition of AMP at pH 6.7 to a Cu^{2+}/ATP system further facilitates the dephosphorylation reaction (Fig. 5). It should be emphasized that neither adenosine (Ado) nor D-ribose 5′-monophosphate ($RibMP^{2-}$), which either lack the phosphate group or the stacking moiety and N-7, are able to promote the reaction; in fact, due to the interaction of these ligands with the metal ion they inhibit the dephosphorylation process.

Most insight into the reaction mechanism is provided, however, by tubercidin 5′-monophosphate ($TuMP^{2-}$). This nucleotide is synthesized by molds and fungi, it has the same self-stacking properties as AMP [50], and it is structurally completely identical with AMP except that N-7 of AMP is replaced by a CH unit (Fig. 7, *vide infra*) (for the metal ion affinity of $TuMP^{2-}$ see ref. 51). As is seen in Fig. 5, addition of TuMP to the Cu^{2+}:ATP 1:1 system inhibits the reaction. This proves unequivocally that N-7 is the crucial binding site for the metal ion already bound to the phosphate group of the other nucleotide as shown in structure C of Fig. 3.

It may be added that the preferred formation of mixed AMP–ATP stacks upon addition of AMP to an ATP system, compared to the formation of pure ATP stacks, is simply a matter of charge repulsion as this is smaller in $[(AMP)(ATP)]^{6-}$ than in $[(ATP)_2]^{8-}$ [2, 3]. With this and the indicated results in mind (for

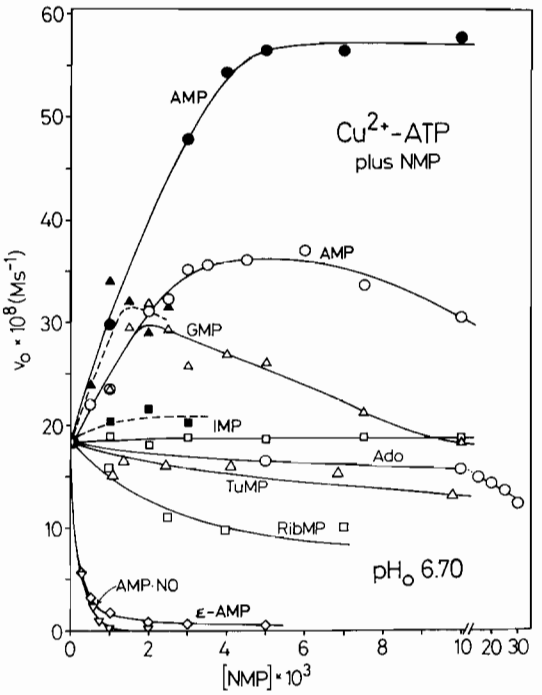

Fig. 5. Influence of purine nucleoside 5′-monophosphates and derivatives (NMP), as well as of Ado and RibMP (on the x axis also designated as NMP) on the initial rate $v_0 = d[PO_4]/dt$ (M s^{-1}) [7] of dephosphorylation of the 1:1 (open symbols ○, △, □, ◇, ▽; $[Cu^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M) and 2:1 (solid symbols ●, ▲, ■; $[Cu^{2+}]_{tot} = 2 \times 10^{-3}$ M and $[ATP]_{tot} = 10^{-3}$ M) Cu^{2+}/ATP systems in aqueous solution at pH_0 6.70: AMP (○, ●), GMP (△, ▲), IMP (□, ■), Ado (○), TuMP (△), RibMP (□), ε-AMP (◇), and AMP·NO (▽). The broken lines indicate uncertainty due to precipitation. $I = 0.1$ M, $NaClO_4$; 50 °C. The above figure combines part of the results shown in Figs. 9 and 10 of ref. 34.

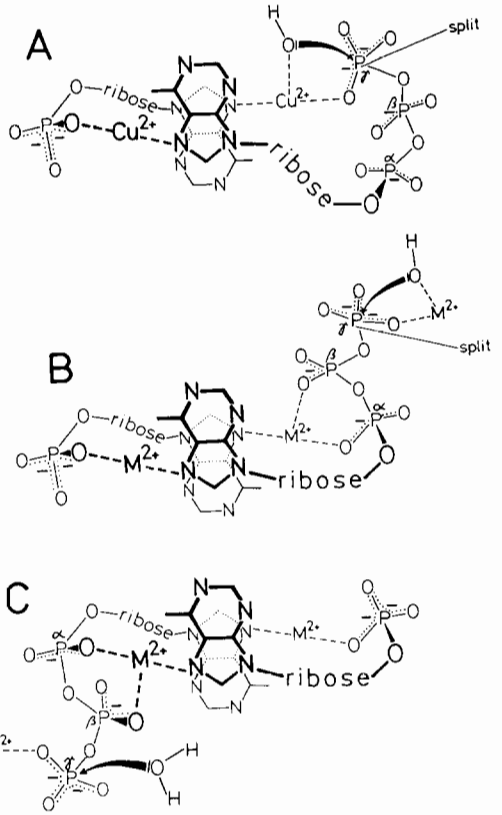

Fig. 6. Comparison of the three types of reactive species which contain an AMP–ATP stack and which are formed during the AMP promoted dephosphorylation of M^{2+}/ATP systems (cf. Fig. 5). A: possible structure of the reactive $Cu_2(ATP)(AMP)(OH)^{3-}$ species (see also the comments given in the footnote* on p. 5). B: probable structure of the reactive $M_3(ATP)(AMP)(OH)^-$ species; the intramolecular attack of OH^- is indicated on the right-hand side, while the left-hand side shows the metal ion bridging and thus stabilizing the purine stack by coordination to the phosphate group of AMP^{2-} and to N-7 of ATP^{4-}. C: probable structure of the reactive $M_3(ATP)(AMP)$ species; in this case the metal ion bridging of the purine stack is indicated at the right-hand side, while the left-hand side undergoes an intermolecular water attack. Note the relations and similarities of the above three structures with the dimers C and D shown in Fig. 3 (see also text).

further details see ref. 7) one may propose the structures shown in Fig. 6 for reactive mixed AMP–ATP stacks formed under various reaction conditions; it is evident that $M_3(ATP)(AMP)$ (Fig. 6C) and $M_3(ATP)(AMP)(OH)^-$ (Fig. 6B) are derived from structure C and $Cu_2(ATP)(AMP)(OH)^{3-}$ (Fig. 6A) from structure D in Fig. 3, respectively.

Most remarkable about this AMP promoted dephosphorylation of ATP, which gives rise to a transphosphorylation (in the simplest case to H_2O), is that the formed products (ADP and finally AMP) are not lost for the reactivity; by their inevitable formation they further promote (and this may also be surmised for ADP) the reactivity of ATP. This is an ideal situation for a reactive compound to allow it to stick out of the chemical 'noise' and to participate in evolution.

Of course, AMP is not the only purine nucleoside 5′-monophosphate which is able to stack and which offers the N-7 position; hence, other nucleotides should also be able to take over the role of the structuring ATP in the dimer (Fig. 3). Indeed, IMP and GMP

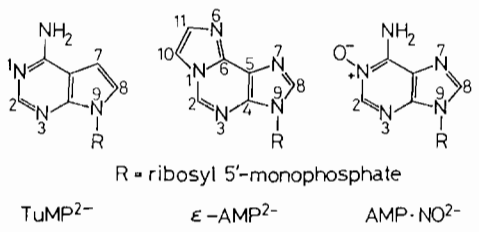

Fig. 7. Chemical structures of some AMP derivatives: $TuMP^{2-}$, tubercidin 5′-monophosphate (=7-deaza-AMP^{2-}); ε-AMP^{2-}, 1,N^6-ethenoadenosine 5′-monophosphate; and $AMP \cdot NO^{2-}$, adenosine 5′-monophosphate N(1)-oxide.

TABLE 2. Solvent influence on the initial rate of dephosphorylation, $v_0 = d[PO_4]/dt$ (M s^{-1}) [7], of M^{2+}/ATP systems ([ATP] = 10^{-3} M) at pH_0 7.50 (I = 0.1 M, $NaClO_4$; 50 °C), together with the solvent promotion factors PF[a, b]

System	Solvent[b]					
	Water		30% Dioxane		50% Dioxane	
	$v_0 \times 10^8$	PF[a]	$v_0 \times 10^8$	PF[a]	$v_0 \times 10^8$	PF[a]
Mg^{2+}/ATP = 1:1	0.045	1.0	0.085	1.9	0.20	4.4
= 2:1	0.050	1.0	0.098	2.0	0.24	4.8
Zn^{2+}/ATP = 1:1	0.25	1.0	0.77	3.1	3.5[c]	14
= 2:1	1.2	1.0	4.1[c]	3.4		
Zn^{2+}/ATP/Mg^{2+} = 1:1:1	0.62	1.0	2.05[c, d]	3.3[d]		

[a]The above results are calculated and in part also abstracted from the data listed in Table 4 of ref. 42. The promotion factors (PF) are based on the rates in water, e.g. $PF = 0.085/0.045 = 1.9$. [b]The 1,4-dioxane content in 30% (vol./vol.) and 50% (vol./vol.) dioxane–water mixtures corresponds to a mol fraction of 0.083 and 0.175, respectively. The dielectric constants for the three solvents are 78.5 (water), 52.7 (water containing 30% dioxane) and 35.2 (water containing 50% dioxane). [c]Toward the end of the experiments partly a slight turbidity appeared. [d]The absolute promotion factor (APF) as defined in footnote 'a' of Table 1 is in this case close to 140.

also promote the dephosphorylation in the Cu^{2+}/ATP system (Fig. 5), yet to a smaller extent which is in accordance with the decreasing self-association tendency in the series $AMP^{2-} > GMP^{2-} > IMP^{2-}$ [3].

Other points to be emphasized are: (i) the orientation of N-7 in the dimer and possibly its binding strength are also crucial; ϵ-AMP and AMP·NO (Fig. 7) very strongly inhibit the dephosphorylation process upon their addition to the Cu^{2+}/ATP system (see Fig. 5); (ii) there is evidence that the described promotion of the ATP dephosphorylation by AMP not only occurs in the presence of Cu^{2+} but also in the presence of Zn^{2+} [7, 34].

A further aspect to be indicated in this connection is that a decreasing solvent polarity does not strongly inhibit the formation of metal ion-bridged stacks [37] while the stability of phosphate metal ion complexes is considerably enhanced [37, 52]. In agreement herewith are the results summarized in Table 2, which do not only confirm the synergistic effects between Mg^{2+} and Zn^{2+} in ATP dephosphorylation but which also show that increasing amounts of 1,4-dioxane added to the aqueous reaction mixture further promote the reactivity of the systems. These observations are meaningful regarding enzymes, as in their active site cavities a lower effective dielectric constant is expected to operate [53–55] compared with that in bulk water; a situation that might correspond also to surfaces like those of clay, which were suggested [33] as being important for the development of life on the early earth.

Phosphate bond cleavage and redox reactions

The results summarized in Fig. 8 show that despite the large formation degree of $Mn(ATP)^{2-}$, Mn^{2+} is a poor promoter of ATP dephosphorylation [7]. However,

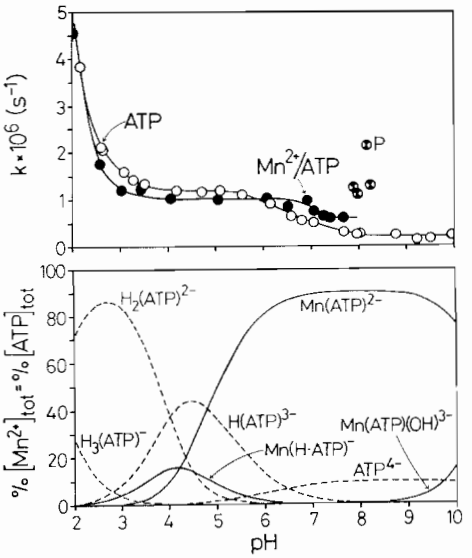

Fig. 8. Upper part: first-order rate constants, k (s^{-1}), for the dephosphorylation of ATP (○) and ATP in the presence of one equivalent Mn^{2+} (●) in aqueous solution as a function of pH [56]; $[ATP]_{tot} = 10^{-3}$ M; I = 0.1 M, $NaClO_4$; 50 °C. Results from solutions in which Mn^{2+} was partially oxidized to Mn^{3+} are also shown (⊛); P indicates precipitation. Lower part: effect of pH at 25 °C (I = 0.1 M, $Na^+ClO_4^-/NO_3^-$) on the concentration of the species present in an aqueous solution of Mn^{2+} and ATP (each 10^{-3} M); the results are given as the percentage of the total Mn^{2+} present (equal to total ATP); the broken lines refer to the free ATP species and the solid lines to the ATP complexes. The results were computed with the following equilibrium constants: $pK^H_{H3(ATP)} = \approx 1.6$, $pK^H_{H2(ATP)} = 4.00 \pm 0.01$, $pK^H_{H(ATP)} = 6.47 \pm 0.01$, $\log K^{Mn}_{Mn(H \cdot ATP)} = 2.74 \pm 0.06$, $\log K^{Mn}_{Mn(ATP)} = 5.01 \pm 0.05$, $pK^H_{Mn(ATP)(H_2O)} = 10.7$. The diprotonated $Mn(H_2 \cdot ATP)$ complex was ignored in the calculations as the corresponding constant is unknown; however, such species would probably exist only below pH 3. (Reproduced from Fig. 14 in ref. 7 (*Coord. Chem. Rev.*, 100 (1990) 453) with permission of Elsevier Science Publishers.)

if air is not completely eliminated in such experiments [56], traces of dioxygen lead at a pH around 8 to oxidation of Mn^{2+} to Mn^{3+} and this latter ion has a much higher affinity toward phosphate groups and it may also carry an OH^- ion at this pH and these points together lead to a sudden 'explosion' of the reactivity. In other words, the dephosphorylation of ATP may be triggered by a redox reaction, in the present case by the oxidation of Mn^{2+} to Mn^{3+}. Similar properties are expected for the Fe^{2+}/Fe^{3+} couple.

In this connection it appears worthwhile to point out a recent observation [57] made with a 'Mn^{2+}-dependent ribozyme'. In this case one RNA hairpin undergoes strand scission at a specific site in the presence of Mn^{2+}. This reaction readily occurs under physiological conditions and it is unaffected by the presence of Mg^{2+}. With the preceding results in mind one wonders if the mentioned strand scission at pH 7.5 is not a result of a partial oxidation of Mn^{2+} to Mn^{3+} which is expected to occur with Mn^{2+} bound to phosphate groups if dioxygen is not rigorously excluded.

Concluding comments

The idea that ATP played a crucial and 'active' role in early evolution is to some extent indirectly confirmed by today's situation. In contemporary biochemistry ATP is still the most important energy-rich intermediate in metabolic processes. The enzyme-catalyzed hydrolysis of ATP to ADP and PO_4 is the main source of energy and Mg^{2+} is known to be essential for this phosphoryl transfer reaction [58, 59]. Indeed, Boyer estimated [60] based on known metabolic pathways and the extent of the world's biomass that ATP, and ADP and PO_4 from which it is formed, participate in more chemical reactions than any other compound on the earth's surface except water. In other words: has ATP simply conserved its eminent role for life over billions of years?

It is extraordinary that ATP can reach extremely high concentrations [61, 62] in certain cell organelles, e.g. the storage vesicles – so called dense bodies – of (human) blood platelets [63] or the chromaffin granules of the (bovine) adrenal medulla [64]. For example in the latter [64] the ATP concentration can be as high as 0.15 M. Considering that there are also significant amounts of metal ions present one could imagine that for example via a redox reaction ($Fe^{2+} \rightarrow Fe^{3+}$/?) a 'bombardment' of transphosphorylations is triggered. Of course such a high nucleotide concentration means that the theoretically high osmotic pressure has to be reduced to isotonicity; this is possible via the well known self-stacking of ATP and related nucleotides [3, 65, 66]; indeed, by ultrazentrifugation [67] the occurrence of high-molecular-weight associates has been proven. One could imagine that the self-stacking is further facilitated by lining up the nucleotide molecules at a protein matrix; in fact, we could recently show that poly-α-lysine promotes the self-association of ATP [68]. This in turn then also means that at early stages in the evolution ATP and related nucleotides could have been lined up at surfaces, for example clay [33, 69] or pyrite [70], possibly with the aid of metal ions and further ligands taking care of the charge situation.

I have mostly focused on ATP in this essay because experimental results are available for this nucleotide [7]. However, much of the prebiotic and evolutionary role which has been attributed here to ATP in an early primitive metabolism without genetics could probably also have been carried out by ADP, which in fact co-exists with AMP and ATP in equilibrium under physiological conditions [71]. In fact, some preliminary observations [72] show that there is also for the metal ion (Cu^{2+}) promoted dephosphorylation of ADP a certain pH range where the rate is proportional to the square of the ADP concentration. This indicates that the dephosphorylation process proceeds also in this case via a dimeric species for which a structure rather close to the one shown in Fig. 3C for ATP can be imagined. This then means that there is also in this case a high probability that the role of one 'structuring' ADP in the dimer can be taken over by AMP. Certainly, whether these ideas are meaningful will finally have to be proven by experiments!

The purine nucleotides ITP and GTP also show a metal ion promoted dephosphorylation (see Fig. 2). Their reaction rates are lower than those of ATP, yet, in a certain pH range also dimeric species are involved [7]. The reason for the smaller reactivity compared to the ATP systems is most probably connected with the smaller self-stacking tendency of ITP and GTP. However, it is easy to imagine that in early evolution, with inosine and guanosine being around [25], the mentioned nucleotides were incorporated into certain processes of a primitive metabolism and in this way paved their path into today's nucleic acids. Considering the 'active' role that purine nucleotides (see also ref. 73) may have played in early evolution, due to the properties of the purine moiety, one is inclined to suggest that the pyrimidines, also available on the primitive earth [25], were incorporated into living systems in a more 'passive' way, possibly even directed by the purines. Finally note, also 'todays' ribozymes [57, 74] depend in their activity on the presence of metal ions.

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References

1 A. Szent-Györgyi, in O. H. Gaebler (ed.), *Enzymes: Units of Biological Structure and Function,* Academic Press, New York, 1956, p. 393.
2 K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs and H. Sigel, *J. Am. Chem. Soc., 103* (1981) 247.
3 H. Sigel, *Biol. Trace Element Res., 21* (1989) 49.
4 H. Sigel, R. Tribolet, R. Malini-Balakrishnan and R. B. Martin, *Inorg. Chem., 26* (1987) 2149.
5 H. Sigel, *Eur. J. Biochem., 165* (1987) 65.
6 H. Sigel, *ACS Symp. Ser., 402* (1989) 159.
7 H. Sigel, Coord. Chem. Rev., 100 (1990) 453.
8 A. I. Oparin, *The Origin of Life on the Earth,* Academic Press, New York, 1957.
9 S. L. Miller and L. E. Orgel, *The Origins of Life on the Earth,* Prentice-Hall, Englewood Cliffs, NJ, 1974.
10 S. F. Mason, *Chemical Evolution: Origin of the Elements, Molecules and Living Systems,* Oxford University Press, Oxford, 1991.
11 M. T. Beck, in H. Sigel (ed.), *Metal Ions in Biological Systems,* Vol. 7, Marcel Dekker, New York, 1978, p. 1.
12 A. W. Schwartz, *Naturwissenschaften, 70* (1983) 373.
13 C. F. Chyba, P. J. Thomas, L. Brookshaw and C. Sagan, *Science, 249* (1990) 366.
14 V. I. Goldanskii and V. V. Kuzmin, *Nature (London), 352* (1991) 114.
15 M. Mitchell Waldrop, *Science, 246* (1989) 1248.
16 J. Horgan, *Sci. Am., 264* (2) (1991) 100.
17 M. Hoffman, *Science, 254* (1991) 379.
18 C. de Duve, *Blueprint for a Cell,* N. Patterson, Burlington, NC, 1991.
19 (a) H. Sigel, *Coord. Chem. Rev., 100* (1990) 453, see p. 496; (b) H. Sigel, in *Abstr., 38th Okazaki Conf.: Structures and Dynamic Aspects of Metal Complexes in Biological Systems,* Institute of Molecular Science, Okazaki, Japan, Oct. 17, 1990.
20 H. Sigel, in *Book of Abstracts of the 33rd IUPAC Congress,* The Hungarian Academy of Sciences, Budapest, 1991, p. 139, no. 426.
21 R. Stribling and S. L. Miller, *Origins Life, 17* (1987) 261.
22 A. W. Schwartz and and C. G. Bakker, *Science, 245* (1989) 1102.
23 J. Oró, *Biochem. Biophys. Res. Commun., 2* (1960) 407.
24 R. A. Sanchez, J. P. Ferris and L. E. Orgel, *J. Mol. Biol., 30* (1967) 223.
25 S. L. Miller and L. E. Orgel, *The Origins of Life on the Earth,* Prentice Hall, Engelwood Cliffs, NJ, 1974, Ch. 8.
26 A. W. Schwartz, A. B. Voet and M. Van der Veen, *Origins Life, 14* (1984) 91.
27 D. C. Mauzerall, *Origins Life Evol. Biosphere, 20* (1990) 293.
28 A. W. Schwartz and M. Goverde, *J. Mol. Evol., 18* (1982) 351.
29 (a) N. W. Gabel and C. Ponnamperuma, *Nature (London), 216* (1967) 453; (b) C. Reid and L. E. Orgel, *Nature (London), 216* (1967) 455.
30 (a) D. Mueller, S. Pitsch, A. Kittaka, E. Wagner, C. E. Wintner and A. Eschenmoser, *Helv. Chim. Acta, 73* (1990) 1410; (b) E. Wagner, Y. B. Xiang, K. Baumann, J. Gueck and A. Eschenmoser, *Helv. Chim. Acta, 73* (1990) 1391; (c) S. Drenkard, J. Ferris and A. Eschenmoser, *Helv. Chim. Acta, 73* (1990) 1373; (d) A. Eschenmoser and M. Dobler, *Helv. Chim. Acta, 75* (1992) 218; (e) A. Eschenmoser and E. Loewenthal, *Chem. Soc. Rev., 21* (1992) 1.
31 S. L. Miller and L. E. Orgel, *The Origins of Life on the Earth,* Prentice-Hall, Englewood Cliffs, NJ, 1974, Ch. 11.
32 M. W. Hosseini and J.-M. Lehn, *J. Chem. Soc., Chem. Commun.,* (1991) 451.
33 A. G. Cairns-Smith, *Sci. Am.,* 252(6) (1985) 74.
34 H. Sigel, F. Hofstetter, R. B. Martin, R. M. Milburn, V. Scheller-Krattiger and K. H. Scheller, *J. Am. Chem. Soc., 106* (1984) 7935.
35 H. Sigel and P. E. Amsler, *J. Am. Chem. Soc., 98* (1976) 7390.
36 (a) C. F. Naumann and H. Sigel, *J. Am. Chem. Soc., 96* (1974) 2750; (b) Y. Fukuda, P. R. Mitchell and H. Sigel, *Helv. Chim. Acta, 61* (1978) 638.
37 R. Tribolet, R. Malini-Balakrishnan and H. Sigel, *J. Chem. Soc., Dalton Trans.,* (1985) 2291.
38 H. Sigel and F. Hofstetter, *Eur. J. Biochem., 132* (1983) 569.
39 V. Scheller-Krattiger and H. Sigel, *Inorg. Chem., 25* (1986) 2628.
40 H. Sigel, *Chimia, 41* (1987) 11.
41 E. R. Werner and B. M. Rode, *Inorg. Chim. Acta, 80* (1983) 39.
42 H. Sigel and R. Tribolet, *J. Inorg. Biochem., 40* (1990) 163.
43 (a) R. J. P. Williams, *Endeavour, 8* (1984) 65; (b) R. J. P. Williams, *Pure Appl. Chem., 55* (1983) 35.
44 (a) B. L. Vallee, *J. Inorg. Biochem., 43* (1991) 84; (b) B. L. Vallee and D. S. Auld, *Biochemistry, 29* (1990) 5656; (c) B. L. Vallee, J. E. Coleman and D. S. Auld, *Proc. Natl. Acad. Sci. U.S.A., 88* (1991) 999.
45 H. Sigel and A. Sigel (eds.), *Zinc and Its Role in Biology and Nutrition, Metal Ions in Biological Systems,* Vol. 15, Marcel Dekker, New York, 1983.
46 H. Sigel and A. Sigel (eds.), *Interrelations Among Metal Ions, Enzymes and Gene Expression, Metal Ions in Biological Systems,* Vol. 25, Marcel Dekker, New York, 1989.
47 J. E. Coleman and D. P. Giedroc, *Met. Ions Biol. Syst., 25* (1989) 171.
48 G. L. Eichhorn, *Met. Ions Biol. Syst., 10* (1980) 1.
49 F. Y.-H. Wu and C.-W. Wu, *Met. Ions Biol. Syst., 15* (1983) 157.
50 R. Tribolet and H. Sigel, *Eur. J. Biochem., 163* (1987) 353.
51 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc., 110* (1988) 6857.
52 G. Liang, N. A. Corfù and H. Sigel, *Z. Naturforsch., Teil B, 44* (1989) 538.
53 H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring and R. Malini-Balakrishnan, *Eur. J. Biochem., 152* (1985) 187.
54 (a) G. R. Moore, *FEBS Lett., 161* (1983) 171; (b) N. K. Rogers, G. R. Moore and M. J. E. Sternberg, *J. Mol. Biol., 182* (1985) 613.
55 D. C. Rees, *J. Mol. Biol., 141* (1980) 323.
56 P. E. Amsler and H. Sigel, *Eur. J. Biochem., 63* (1976) 569.
57 V. Dange, R. B. Van Atta and S. M. Hecht, *Science, 248* (1990) 585.
58 K. Yoshikawa, Y. Shinohara, H. Terada and S. Kato, *Biophys. Chem., 27* (1987) 251.
59 A. S. Mildvan, *Magnesium, 6* (1987) 28.
60 P. D. Boyer, *Biochemistry, 26* (1987) 8503.
61 N. A. Thorn, J. T. Russell and M. Treiman, in A. M. Poisner and J. M. Trifaró (eds.), *The Secretory Granule,* Elsevier, Amsterdam, 1982, pp. 119–151.
62 (a) K. S. Rajan, R. W. Colburn and J. M. Davis, *Met. Ions Biol. Syst., 6* (1976) 291; (b) R. W. Colburn and J. W. Maas, *Nature (London), 208* (1965) 37.

63 H. Holmsen and H. J. Day, *Ser. Haematol., IV* (1) (1971) 28.

64 H. Winkler and S. W. Carmichael, in A. M. Poisner and J. M. Trifaró (eds.), *The Secretory Granule,* Elsevier, Amsterdam, 1982, pp. 3–79.

65 N. A. Corfù, R. Tribolet and H. Sigel, *Eur. J. Biochem., 191* (1990) 721.

66 N. A. Corfù and H. Sigel, *Eur. J. Biochem., 199* (1991) 659.

67 A. Pletscher, M. Da Prada, K. H. Berneis, H. Steffen, B. Lütold and H. G. Weder, *Adv. Cytopharmacol., 2* (1974) 257.

68 H. Sigel and N. A. Corfù, results to be published.

69 (a) A. G. Cairns-Smith, *Genetic Takeover and the Mineral Origins of Life,* Cambridge University Press, Cambridge, UK, 1982; (b) A. G. Cairns-Smith, *Int. Rev. Phys. Chem., 7* (1988) 209.

70 (a) G. Wächtershäuser, *Microbiol. Rev., 52* (1988) 452; *Proc. Natl. Acad. Sci. U.S.A., 87* (1990) 200; (b) E. Drobner, H. Huber, G. Wächtershäuser, D. Rose and K. O. Stetter, *Nature (London) 346* (1990) 742.

71 (a) R. N. Goldberg and Y. B. Tewari, *Biophys. Chem., 40* (1991) 241; (b) Y. B. Tewari, R. N. Goldberg and J. V. Advani, *Biophys. Chem., 40* (1991) 263.

72 H. Sigel and S. S. Massoud, unpublished observations and results to be published.

73 G. Wächtershäuser, *Proc. Natl. Acad. Sci. U.S.A., 85* (1988) 1134.

74 J.-P. Perreault, D. Labuda, N. Usman, J.-H. Yang and R. Cedergren, *Biochemistry, 30* (1991) 4020; P. S. Freemont, J. M. Friedman, L. S. Beese, M. R. Sanderson and T. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A., 85* (1988) 8924, see p. 8927.