Hydrolysis of Nucleoside Phosphates, III [1, 2]

The Dephosphorylation of Adenosine 5′-Triphosphate in a Binary and Ternary Zn^{2+} Complex

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The dephosphorylation of ATP was characterized by determining the dependence of the first-order rate constant on pH in the absence and presence of Zn^{2+} and together with Zn^{2+} and 2,2′-bipyridyl. The Zn^{2+}-accelerated reaction passes through a pH optimum at about 8. The decrease in the rate at higher pH is due to the formation of $Zn(ATP)(OH)^{3-}$; this species is relatively insensitive towards dephosphorylation. It is concluded that $Zn(ATP)^{2-}$ is the reactive species and that the interaction between N(7) and Zn^{2+} in this complex is crucial for its reactivity. In the presence of 2,2′-bipyridyl (Bipy) the ternary complex, $Zn(Bipy)(ATP)^{2-}$, is formed which is rather stable towards dephosphorylation. It is suggested that the described effects of acceleration and inhibition are helpful for understanding the recycled processes in nature.

As the transfer of a phosphoryl group is a widespread biological reaction which occurs commonly in the presence of metal ions, it is understandable that the metal ion-catalyzed dephosphorylation of ATP has received attention for several years. The catalysis best studied so far, is that by Cu^{2+} (*cf.* [1–4]). For this metal ion it was shown that interaction occured not only with the phosphate chain, but also with N(7) of the adenine moiety, thus creating a macrochelate, which is indeed crucial for the Cu^{2+}-accelerated dephosphorylation [2, 4]. If the N(7)-interaction is blocked by the formation of mixed-ligand complexes with 2,2′-bipyridyl (Bipy), imidazole or hydroxyl ions, the dephosphorylation is inhibited [2]. This is especially significant in the case of $Cu(Bipy)(ATP)^{2-}$, where the dephosphorylation rate at pH ≤ 7 is even lower than in free ATP [1, 2].

One of the more relevant metal ions involved in biological phosphorylation reactions is Zn^{2+}. In fact, it is known that this metal ion accelerates the dephosphorylation of ATP also [3, 4]. We have now reinvestigated the binary Zn^{2+}-ATP system for reasons of comparison with the ternary system, 2,2′-bipyridyl-Zn^{2+}-ATP, and have shown that in the latter system ATP is protected towards hydrolysis. This result is certainly of interest with regard to the transport of hydrolysis-sensitive phosphates and the mechanisms utilizing the chemical energy of nucleotides in biological systems.

Materials and Methods

The disodium salt of adenosine 5′-triphosphate was purchased from Sigma Chemical Co., St. Louis, Mo., and from Serva Feinbiochemica GMBH, Heidelberg, Germany. Zinc(II) and sodium perchlorate and 2,2′-bipyridyl were from Fluka AG, Buchs, Switzerland.

All experimental procedures were carried out exactly as described recently in detail [2]. The pH in the reaction solutions was adjusted by dotting with NaOH or $HClO_4$. [ATP] at the time, *t*, was determined by the equation, $[ATP]_t = [ATP]_0 - [PO_4]_t$, where $[ATP]_0$ is the initial concentration of ATP, while $[ATP]_t$ and $[PO_4]_t$ are the concentrations at the time, *t*. $[PO_4]$ represents the concentration of all the species H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}. The free phosphate initially present (about 3%) was taken into account in the calculations. The first-order rate constant, k [s^{-1}], was determined from the slope of the straight line portion of a log $[ATP]_t$-time plot. The corresponding pH was obtained by averaging the pH values measured for those samples that gave points on the straight-line portion (*cf* [2]).

Results and Discussion

The rate of dephosphorylation at 50 °C ($I = 0.1$, $NaClO_4$) in the systems, ATP, Zn^{2+}-ATP, and

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2,2′-bipyridyl-Zn^{2+}-ATP is shown in dependence on pH in the upper part of Fig. 1. The Zn^{2+}-catalyzed dephosphorylation shows a maximum at pH 8 with an acceleration of about 15, compared with free ATP. Tetas and Lowenstein [3] observed at 80 °C and in the presence of buffers a similar order of acceleration but with a maximal rate at pH 5. The study by Schneider and Brintzinger [4] at 50 °C is even less comparable with ours, as the Zn^{2+} : ATP ratio was 2 : 1. However, Zn^{2+} definitely increases the hydrolysis of ATP less than does Cu^{2+}, which shows in the maximum a 300 fold rate increase compared to the simple ATP system [2]. This difference may possibly result from a weaker interaction between N(7) and Zn^{2+} [5], even though such an interaction is definitely known [6, 7], and the formation of the resulting macrochelate is also crucial for the enhanced dephosphorylation by Zn^{2+}.

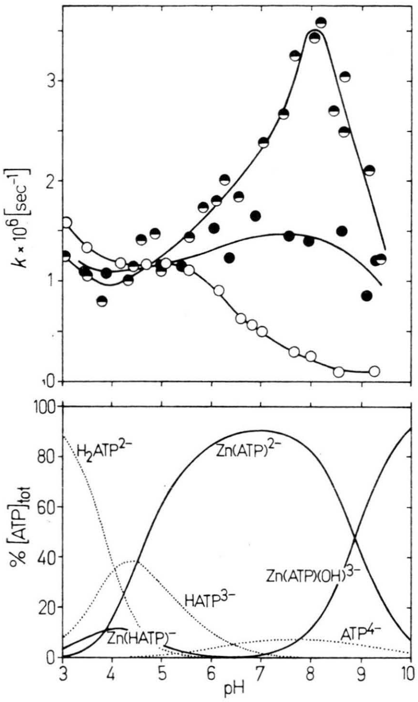

Fig. 1. *Upper part:* Dephosphorylation of ATP under several conditions in dependence on pH, characterized as the first-order rate constant, *k*: ATP alone (○), ATP in the presence of Zn^{2+} (◓) and of Zn^{2+} and Bipy (●). The concentration of all reagents was 10^{-3} M; $I=0.1$, $NaClO_4$; 50 °C. — *Lower part:* Influence of pH on the concentrations (given as the percentage of the total ATP, or Zn^{2+}, present) of the several species present in an aqueous solution at 25 °C ($I=0.1$) of the binary system, Zn^{2+} and ATP (each 10^{-3} M); computed with the constants of refs [8, 13] and yet unpublished data. The dotted lines are due to the free ATP species and the solid lines are due to the ATP complexes.

Most interestingly in the mixed-ligand 2,2′-bipyridyl-Zn^{2+}-ATP system, where the ternary complex $Zn(Bipy)(ATP)^{2-}$ is formed to more than 75% of the total ATP present, in the pH range 6.5 to 9.0, the hydrolysis is inhibited. This corresponds to the observation made with $Cu(Bipy)(ATP)^{2-}$ (*cf.* [2]). In this latter complex the coordination of Cu^{2+} to N(7) is inhibited [8, 9], while a charge-transfer interaction occurs between the purine moiety and 2,2′-bipyridyl, *i.e.* this ternary complex can be considered as a metal ion-bridged charge-transfer complex [9, 10]. An analog structure may be surmised for $Zn(Bipy)(ATP)^{2-}$.

In the lower part of Fig. 1 the distribution of the several species present in the binary system, Zn^{2+}-ATP, is shown. It is quite obvious that the increasing concentration of $Zn(ATP)(OH)^{3-}$ corresponds to the decreasing reaction rate of the upper part of Fig. 1. Hence, one may conclude that in $Zn(ATP)(OH)^{3-}$ the N(7) interaction is nonexistent like in $Cu(ATP)(OH)^{3-}$ (*cf.* [2]). The fact that $Zn(ATP)^{2-}$ has reached high concentrations already at pH about 6 while the maximal rate is observed only at pH about 8, indicates that the reaction rate is not only dependent on the concentration of the binary complex but also on the concentration of OH^-. Probably, the hydrolysis may occur with both, OH^- and H_2O, the former species being somewhat more reactive. However, as $Zn(ATP)(OH)^{3-}$ is rather stable the OH^- attack on the γ-phosphate group [2, 11] in $Zn(ATP)^{2-}$ occurs probably "*trans*" to the coordinated metal ion and not through an intramolecular attack by bound OH^-. This proposed mechanism is in contrast to one suggested by Spiro *et al.* [12] based on the species $[Zn(ATP)(OH)]_2^{6-}$, which can however not be correct as such a hydrolyzed complex cannot yet exist (if it exists at all) in the pH range in question.

Overall then the preceeding results demonstrate unequivocally that Zn^{2+} accelerates the hydrolysis of ATP in the corresponding binary complex, while in its ternary complexes the dephosphorylation is inhibited. These observations appear to be helpful for understanding biological phenomena, *i.e.* the transport and activation of phosphates, as well as cycling processes.

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