involved in bonding through the d orbitals of the vanadium nucleus.

Lactate is a more strongly electron withdrawing group than ethylene glycol. The bipyramidal product formed with lactate is now not electron-rich, and anhydride formation does not occur; indeed, there is a tendency to accept more electrons by expanding the coordination shell by addition of water. This leads to an octahedral product that now is electron-rich. This compound reduces the electron density by anhydride formation accompanied by uptake of a single proton. A further proton can be taken up in a subsequent reaction.

Oxalate is a strongly electron withdrawing group. No bipyramidal product is observed with this ligand. Formation of the octahedral product is accompanied by protonation, but a futher decrease in electron density through anhydride formation is not observed. In this case incorporation of a second oxalate accompanied by uptake of a further proton can and does occur, leading to the major product.

If this description of coordination geometry in terms of electron availability is valid, then it can be predicted that if highly electron withdrawing alcohols are used with vanadate in an effort to form tetrahedral derivatives, bipyramidally or even octahedrally coordinated products may be formed. Sufficiently electron withdrawing diols may lead to the formation of bipyramidal monomeric and octahedral forms as well as the expected bipyramidal anhydrides. Similarly, α-hydroxy acids containing electron-withdrawing groups may form only minimal proportions of bipyramidal products. Conversely, the presence of electron-donating substituents should force the equilibria toward less substituted products.

Acknowledgment. Thanks are gratefully extended to the Natural Sciences and Engineering Research Council of Canada for its support of this work (A.S.T. and M.J.G.), to the B. C. Heart Foundation (M.J.G.), and to the Medical Research Council of Canada (M.J.G.).

Supplementary Material Available: Listings of concentrations of various vanadate species determined as a function of total vanadate and of oxalate concentration (Tables 1s and 2s) and various plots concerning vanadate complex formation (Figures 1s–10s) (12 pages). Ordering information is given on any current masthead page.

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Ternary Complexes in Solution.[1,2] Intramolecular Equilibria in Metal Ion Complexes of Adenosine 5′-Triphosphate (ATP^{4-}): Coordination of Ammonia or Imidazole to $M(ATP)^{2-}$ Releases N-7 from the Metal Ion Coordination Sphere

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Received August 28, 1986

By ^1H NMR shift measurements in D_2O at 27 °C under conditions where the monomeric complexes dominate, it is shown by monitoring the shifts of H-2, H-8, and H-1′ of $Cd(ATP)^{2-}$ and $Zn(ATP)^{2-}$ (ATP^{4-} = adenosine 5′-triphosphate) that upon formation of ternary complexes with ammonia or imidazole N-7 is released from the coordination sphere of the metal ions. These results together with earlier observations show that mixed-ligand complex formation between $M(ATP)^{2-}$ and a unidentate (e.g., OH^-, NH_3, imidazole) or bidentate ligand (e.g., 2,2′-bipyridyl, tryptophanate) leads to release of the adenine moiety from the coordination sphere. A careful analysis of stability data for ternary systems consisting of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}/ATP, UTP/imidazole, OH^- confirms the results obtained by NMR spectroscopy. For $Ni(ATP)^{2-}$ and $Cu(ATP)^{2-}$ the analysis shows that addition of imidazole at least reduces (and possibly also eliminates) the extent of N-7 back-bonding. These results are utilized to calculate the percentage (or its lower limit) of the macrochelated isomer of several $M(ATP)^{2-}$ complexes by employing the difference in complex stability between the ternary $M(ATP)(imidazole)^{2-}$ and $M(UTP)(imidazole)^{2-}$ complexes (UTP^{4-} = uridine 5′-triphosphate). This evaluation is possible because in $M(UTP)^{2-}$ no metal ion/base interaction occurs, allowing an easier access of imidazole (or other ligands, such as NH_3 and OH^-) to the metal ion than in $M(ATP)^{2-}$, where in the back-bound isomer N-7 has to be substituted. The probable consequences of such structural alterations for enzymic systems are briefly indicated.

Adenosine 5′-triphosphate (ATP^{4-}) in the form of metal ion complexes is the substrate of many enzymic reactions.[4] Evidently, during the reaction process at least, ternary complexes of the kind enzyme/metal ion (M^{2+})/nucleotide must be formed.[4-6] Consequently, mixed-ligand complexes of ATP are receiving increasing attention.[7-12]

For the metal ions of the second half of the first transition series and Zn^{2+}, as well as Cd^{2+}, it is known[13,14] that in the binary $M(ATP)^{2-}$ complexes considerable base back-binding occurs.[15] Thus, a significant amount of these complexes contains in equilibrium a species in which the metal ion is bound not only to the triphosphate chain but also to N-7 of the adenine moiety, thus forming a macrochelate. A question arises upon ternary complex formation in such systems: Does this metal ion/N-7 interaction still exist in mixed-ligand complexes? For bidentate ligands such as 2,2′-bipyridyl, 1,10-phenanthroline, and tryptophanate, N-7

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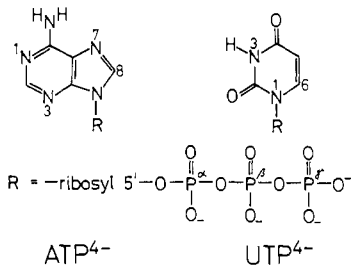


Figure 1. Structures of the nucleoside 5′-triphosphates (NTP^{4-}) used in this study.

release is well-known from studies of the corresponding ternary M^{2+}/ATP complexes in solution[16] and in the solid state.[17] Also, for OH^- as ligand, N-7 release has already been proven by ^1H NMR shift experiments with $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$: in $Zn(ATP)(OH)^{3-}$ and $Cd(ATP)(OH)^{3-}$ N-7 is no longer in the coordination sphere of the metal ions.[14]

As imidazole and amino residues are important binding sites in nature,[18] especially in proteins,[19] we have now studied by ^1H NMR shift experiments the simplest kind of the corresponding mixed-ligand M(ATP)(L) complexes, i.e., those containing ammonia (NH_3) or imidazole (Im). Indeed, it will be shown that even these simple monodentate ligands substitute for N-7 of the adenine moiety rather than for a water molecule.

This result could be further exploited by comparing the stability differences[20] between the ternary complexes M(UTP)(L) and M(ATP)(L) (UTP^{4-} = uridine 5′-triphosphate). The difference in stability reflects the extent of base back-binding in the binary $M(ATP)^{2-}$ complexes, as in the binary complexes with UTP^{4-} (Figure 1); i.e. in $M(UTP)^{2-}$, no base back-binding occurs and the structures of the coordination spheres in the two kinds of ternary complexes are identical. On this basis the formation degree of the macrochelated isomer in several $M(ATP)^{2-}$ complexes is also calculated.

Experimental Section

Materials. The disodium salt of adenosine 5′-triphosphate and the trisodium salt of uridine 5′-triphosphate (both "reinst", research grade) were purchased from Serva Feinbiochemica GmbH, Heidelberg, FRG. The content of free orthophosphate (determined as described in ref 21) was 2.0 and 2.3%, respectively. The nitrate salts of NH_4^+, Na^+, Zn^{2+}, and Cd^{2+}, imidazole, HNO_3, and NaOH (all p.A.), DNO_3 and NaOD (both with >99% D), and a 10% tetramethylammonium hydroxide solution (p.A., which was converted into the nitrate) were obtained from Merck AG, Darmstadt, FRG. D_2O (≥99.8%) was from CIBA-Geigy AG, Basel, Switzerland.

^1H NMR Shift Measurements. The ^1H NMR spectra were recorded with a Bruker WH-90 FT spectrometer (90.025 MHz) in D_2O at 27 °C, with the center peak of the tetramethylammonium ion triplet used as internal reference. All chemical shifts were converted to a 3-(trimethylsilyl)propanesulfonate reference by adding 3.188 ppm. The reliability of tetramethylammonium ion as an internal ^1H NMR reference in such studies has been discussed previously in detail.[13,22] Indeed, the affinity of $(CH_3)_4N^+$ toward di- and triphosphate is very low.[23]

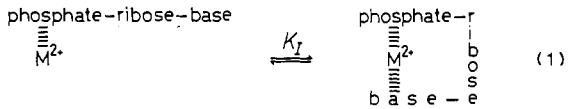
The pD of the solutions was obtained by adding 0.40 to the pH meter reading.[24] The pH was measured with a Metrohm 605 potentiometer (Metrohm AG, Herisau, Switzerland) using a Metrohm EA 125 glass electrode. The desired pD of a solution was adjusted by dotting with relatively concentrated DNO_3 or NaOD on a thin glass rod; the volume changes were negligible.

Further experimental details are given in the legends for the corresponding figures.

Results and Discussion

Indications that the formation of such a simple ternary complex as $M(ATP)(OH)^{3-}$ is connected with a release of N-7 (see Figure 1) from the coordination sphere of the metal ion had first been obtained from studies of the metal-ion-promoted dephosphorylation of nucleoside 5′-triphosphates.[21,25] ^1H NMR experiments with $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ and observation of the chemical shift dependence on pH have confirmed[14] these indications. As ^1H NMR proved ideal for characterizing the structure of the mentioned hydroxo complexes in solution, the same tool was now employed for studying $M(ATP)(NH_3)^{2-}$ and $M(ATP)(Im)^{2-}$ complexes.

1. Influence of NH_3 Coordination to $Cd(ATP)^{2-}$ on the ^1H NMR Shifts for H-2, H-8, and H-1′ of ATP. The reasoning behind these experiments is the earlier observation[13,14] that coordination of a metal ion to N-7 gives rise to the intramolecular equilibrium (1)



(1)

and leads to a downfield shift of the resonance signals of the neighboring H-8. $Cd(ATP)^{2-}$ was selected for the present experiments since in this case the downfield shift is quite pronounced.[13,14] NH_3 was chosen because this ligand is small and is not expected to undergo interactions with ATP in the absence of metal ions, an assumption confirmed by the results.

The results of the ^1H NMR experiments of the $Cd(ATP)^{2-}$/NH_4NO_3 system at pD 9.2 are shown in the upper part of Figure 2. From the left side in this figure (at $[NH_4NO_3] = 0$) it is clear that the interaction of Cd^{2+} with N-7 in $Cd(ATP)^{2-}$ leads to the expected downfield shift of H-8, compared with the shift position of H-8 for uncomplexed ATP^{4-}. The corresponding comparison for H-1′ of the ribose moiety indicates that complex formation with Cd^{2+} has almost no influence while for H-2 an upfield shift is observed due to the known[13] Cd^{2+}-promoted self-stacking.[26] The shift positions shown in Figure 2 for $Cd(ATP)^{2-}$ agree well with earlier measurements.[13,14] In addition, the chemical shifts of ATP^{4-} and $Mg(ATP)^{2-}$ are very similar in dilute solutions,[13] indicating that the influence of charge on the shifts of H-2, H-8, and H-1′ is minor.

For the present, the crucial point is the mentioned downfield shift of H-8 due to the N-7 coordination because this shift in turn means that a release of N-7 in connection with the formation of the ternary $Cd(ATP)(NH_3)^{2-}$ complex should give rise to an upfield shift. Indeed, such is the case (Figure 2A): with increasing concentration of NH_4NO_3 the shift of H-8 for $Cd(ATP)^{2-}$ moves upfield.

The calculated distribution of complex species in the Cd^{2+}/ATP/NH_4^+ system at pD 9.2 depends on the NH_4NO_3 concentration as shown in the lower part of Figure 2. Considering the uncertainty due to the use of equilibrium constants determined for H_2O as solvent[14,15,20] in the calculations[27] for D_2O solutions (see legend for Figure 2B), and considering the fact that the (slight) self-association already present in 5×10^{-3} M $Cd(ATP)^{2-}$

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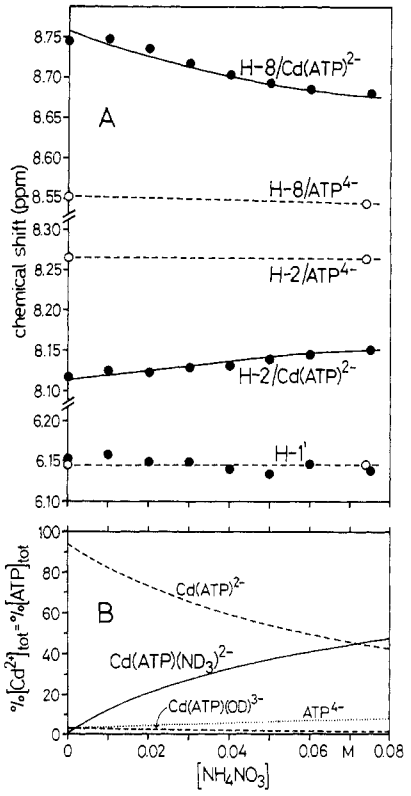

Figure 2. Comparison of (A) the chemical shifts of $Cd(ATP)^{2-}$ under the influence of increasing concentrations of NH_4NO_3 with (B) the resulting increasing concentration of the ternary $Cd(ATP)(NH_3)^{2-}$ complex in D_2O at pD 9.2. (A) Dependence of the chemical shifts of H-2, H-8, and H-1′ of $Cd(ATP)^{2-}$ (●; $[Cd^{2+}]_{tot} = [ATP]_{tot} = 5 \times 10^{-3}$ M; formation degree of $Cd(ATP)^{2-}$ about 95%, see below in (B)) on increasing concentrations of NH_4NO_3 in D_2O at pD 9.2 ($I = 0.1$ ($NaNO_3$); 27 °C). The three nearly horizontal, broken lines represent the chemical shifts of H-2, H-8, and H-1′ of uncomplexed ATP^{4-} (5×10^{-3} M) also under the influence of increasing concentrations of NH_4NO_3 at pD 9.2 (○). The spectra were measured on a Bruker FT-90 instrument at 90.025 MHz, relative to internal $(CH_3)_4N^+$, and converted to values relative to sodium 3-(trimethylsilyl)propanesulfonate by adding 3.188 ppm. (B) Effect of increasing concentrations of NH_4NO_3 (pD 9.2; $I = 0.1$; 25 °C) on the concentration of the species present in a D_2O solution of Cd^{2+} and ATP (each 5×10^{-3} M). The results were computed with the constants listed below, and they are given as the percentage of the total Cd^{2+} present (=total ATP). The slight self-association[26] is neglected, and it is assumed in the calculations that the change from H_2O to D_2O does not influence the size of the stability constants of the complexes: log $K^{Cd}_{Cd(NH_3)} = 2.67$,[20] log $K^{Cd(NH_3)}_{Cd(NH_3)_2} = 2.27$ (estimate), log $K^{Cd}_{Cd(H\cdot ATP)} = 3.04$,[15] log $K^{Cd}_{Cd(ATP)} = 5.34$,[15] log $K^{Cd(ATP)}_{Cd(ATP)(NH_3)} = 2.0 \pm 0.2$.[20] The acidity constants measured in H_2O (these values and their source are given in parentheses) were transformed with a published relation[27] in values valid for D_2O as solvent: $pK^D_{ND_4} = 9.97$ (9.38[20]), $pK^D_{D_2(ATP)} = 4.51$ (4.00[15]), $pK^D_{D(ATP)} = 7.02$ (6.47[15]), $pK^D_{Cd(ATP)(D_2O)} = 10.7$ (10.1[14]).

solutions[26] was neglected, the correspondence between the upper and lower parts of Figure 2 is excellent. The formation degree of $Cd(ATP)(ND_3)^{2-}$ reaches at $[NH_4NO_3] = 0.075$ M (Figure 2B) about 45%, and the upfield shift observed for H-8 under the corresponding conditions is about 40% (Figure 2A). Hence, upon coordination to $Cd(ATP)^{2-}$, NH_3 substitutes for N-7 of the adenine residue rather than for a water molecule; in other words, N-7 is released from the coordination sphere of the metal ion upon formation of $Cd(ATP)(NH_3)^{2-}$.

The shift of H-2 moves upfield (Figure 2A) by about 25% under the above conditions. This percentage is in line with the expected reduced self-association tendency of the ternary $Cd^{2+}/ATP/NH_4^+$ system compared with the binary Cd^{2+}/ATP system. Cd^{2+} promotes the self-stacking of ATP^{4-} by forming intermolecular links between ATP^{4-} species in dimer stacks: i.e., by coordinating to the phosphate residue of one ATP^{4-} and to N-7 of the neighboring ATP^{4-} (for details see ref 13) and also by reducing the

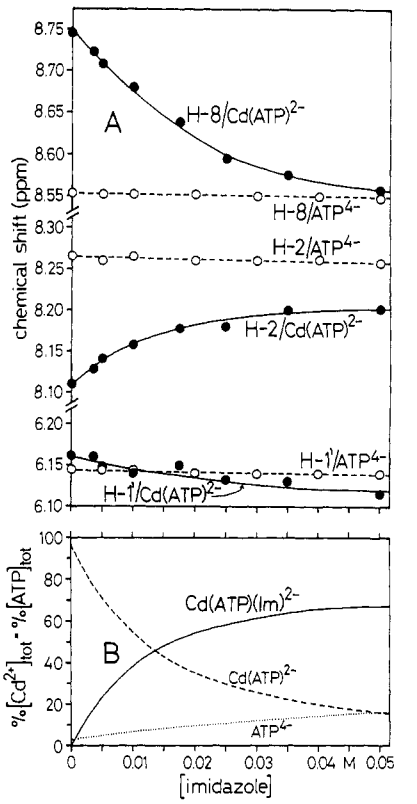

Figure 3. Comparison of (A) the chemical shifts of $Cd(ATP)^{2-}$ under the influence of increasing concentrations of imidazole with (B) the resulting increasing concentration of the ternary $Cd(ATP)(Im)^{2-}$ complex in D_2O at pD 8.4. (A) Dependence of the chemical shifts of H-2, H-8, and H-1′ of $Cd(ATP)^{2-}$ (●; $[Cd^{2+}]_{tot} = [ATP]_{tot} = 5 \times 10^{-3}$ M; formation degree of $Cd(ATP)^{2-}$ about 96%, see below in (B)) on increasing concentrations of imidazole in D_2O at pD 8.4 ($I = 0.1$ ($NaNO_3$); 27 °C). The three nearly horizontal, broken lines represent the chemical shifts of H-2, H-8, and H-1′ of uncomplexed ATP (5×10^{-3} M) also at pD 8.4 and under the influence of increasing concentrations of imidazole (○) (see also legend to Figure 2). (B) Effect of increasing concentrations of imidazole (pD 8.4; $I = 0.1$; 25 °C) on the concentration of the species present in a D_2O solution of Cd^{2+} and ATP (each 5×10^{-3} M). The results are plotted as in Figure 2 ($[Cd(ATP)(OD)^{3-}] < 0.5\%$); they were computed with those constants given in the legend of Figure 2, which are also valid here, and the additional constants[26] log $K^{Cd}_{Cd(Im)} = 2.71$,[20] log $K^{Cd(Im)}_{Cd(Im)_2} = 2.31$ (estimate), log $K^{Cd(ATP)}_{Cd(ATP)(Im)} = 2.03$,[20] and $pK^D_{D(Im)} = 7.60$ (7.04).[20]

charge repulsion in the stacks by neutralizing part of the negative charge of the phosphate residues through coordination. The latter effect will still operate in a $Cd(ATP)(NH_3)^{2-}$ system while the former one will be reduced. H-1′ is not influenced by coordination of Cd^{2+} to ATP^{4-}, and therefore the release of N-7 is also not reflected in the chemical shift of this proton (Figure 2A).

The corresponding experiments with $Zn(ATP)^{2-}$ and NH_3 are unfortunately not possible, because hydroxo complex formation of $Zn(ATP)^{2-}$ already interferes strongly at pD 9.2 ($pK^D_{Zn(ATP)(D_2O)} = 9.45$; see legend to Figure 4B).[28] This is also the reason that for $Zn(ATP)(NH_3)^{2-}$ only a limiting value for the stability constant had been determined earlier.[20]

2. Influence of Imidazole Coordination on the ^1H NMR Shifts of $Cd(ATP)^{2-}$ and $Zn(ATP)^{2-}$. Experiments with $Cd(ATP)^{2-}$ and $Zn(ATP)^{2-}$ employing increasing amounts of imidazole were carried out at pD 8.4; the corresponding ^1H NMR shift values are plotted in Figures 3 and 4, respectively. The chemical shifts of the protons of uncomplexed ATP^{4-} are hardly affected by increasing amounts of imidazole.

The downfield shift observed for H-8 of the binary $M(ATP)^{2-}$ complexes is significantly more pronounced with Cd^{2+} than with Zn^{2+} (see the left side in Figures 3 and 4 where [Im] = 0); this

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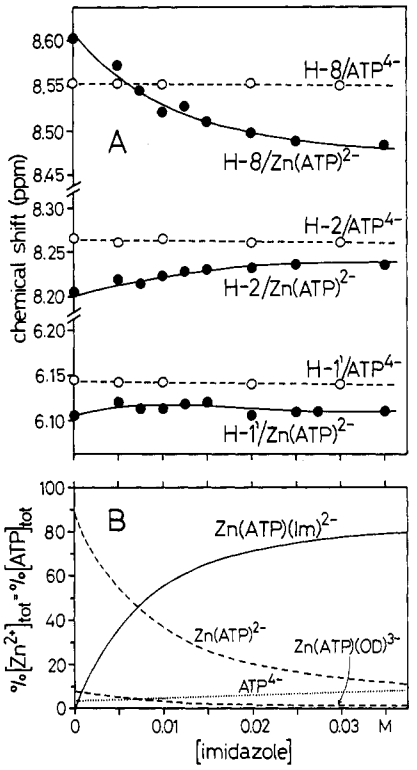

Figure 4. Comparison of (A) the chemical shifts of $Zn(ATP)^{2-}$ under the influence of increasing concentrations of imidazole with (B) the resulting increasing concentration of the ternary $Zn(ATP)(Im)^{2-}$ complex in D_2O at pD 8.4. (A) Dependence of the chemical shifts of H-2, H-8, and H-1′ of $Zn(ATP)^{2-}$ (●; $[Zn^{2+}]_{tot} = [ATP]_{tot} = 5 \times 10^{-3}$ M; formation degree of $Zn(ATP)^{2-}$ close to 90%, see below in (B)) on increasing concentrations of imidazole in D_2O at pD 8.4 ($I = 0.1$ ($NaNO_3$); 27 °C). The three nearly horizontal, broken lines represent the chemical shifts of H-2, H-8, and H-1′ of uncomplexed ATP (5×10^{-3} M) also at pD 8.4 and under the influence of increasing concentrations of imidazole (○) (see also legend to Figure 2). (B) Effect of increasing concentrations of imidazole (pD 9.2; $I = 0.1$; 25 °C) on the concentration of the species present in a D_2O solution of Zn^{2+} and ATP (each 5×10^{-3} M). The results are plotted as in Figure 2; they were computed with those constants given in the legend of Figure 2, which are also valid here, and the additional constants[26] log $K^{Zn}_{Zn(Im)} = 2.51$,[20] log $K^{Zn(Im)}_{Zn(Im)_2} = 2.11$ (estimate), log $K^{Zn}_{Zn(H\cdot ATP)} = 2.86$,[15] log $K^{Zn}_{Zn(ATP)} = 5.16$,[15] log $K^{Zn(ATP)}_{Zn(ATP)(Im)} = 2.41$;[20] $pK^{D}_{D(Im)} = 7.60$ (7.04),[20] and $pK^{D}_{Zn(ATP)(D_2O)} = 9.45$ (8.87).[28]

observation was made before[14] and agrees with the higher formation degree of the macrochelated $M(ATP)^{2-}$ isomer in the Cd^{2+} complex.[15] Addition of imidazole to $M(ATP)^{2-}$ induces again a significant upfield shift for H-8 in both systems, confirming that N-7 is also released in $M(ATP)^{2-}$ upon formation of $Cd(ATP)(Im)^{2-}$ (Figure 3A) and $Zn(ATP)(Im)^{2-}$ (Figure 4A).

However, comparison of the extent of the aforementioned upfield shifts for H-8 with the formation degrees of the ternary complexes as shown in the lower parts of Figures 3 and 4 indicates that these upfield shifts must have one further source. This is especially evident from the $Zn(ATP)^{2-}$/Im system (Figure 4A), where the upfield shift of H-8 proceeds significantly beyond the shift position of H-8 in uncomplexed ATP (which at pD 8.4 is largely present as ATP^{4-}; $pK^{D}_{D(ATP)} = 7.02$, see legend to Figure 2B). This additional upfield shift suggests that coordination of imidazole to $M(ATP)^{2-}$ obviously has also a shielding effect on H-8; most probably in $M(ATP)(Im)^{2-}$ also some intramolecular stacking between the coordinated imidazole and the purine system of ATP occurs. That the imidazole ring may undergo stacking[9b,29] and that bridging by a metal ion facilitates stacking between otherwise only weakly interacting aromatic-ring systems have been observed before.[2,8b,11,12,16] Indeed, the upfield shift observed for H-2, H-8, and H-1′ of ATP^{4-} upon addition of imidazole is minute, indicating that the stability of the binary, unbridged stack is very low.

The chemical shift of H-1′ of the ribose moiety is somewhat affected at pD 8.4 upon coordination of Cd^{2+} or Zn^{2+} to ATP^{4-}, and coordination of imidazole to $M(ATP)^{2-}$ also has a slight effect (Figures 3 and 4). Regarding H-2, the points outlined in part 1 also apply here, though the downfield shifts of about 60% and 65% for $Cd(ATP)^{2-}$/Im and $Zn(ATP)^{2-}$/Im respectively correspond rather well to the formation degrees of about 67% (Figure 3B) and 80% (Figure 4B) of the ternary $M(ATP)(Im)^{2-}$ complexes, thus supporting the conclusion on the release of N-7 upon formation of the mixed-ligand complexes.

Indications for the release of the adenine moiety from the coordination sphere of the metal ion through the additional coordination of imidazole were obtained earlier[30] from kinetic experiments. The Cu^{2+}-promoted hydrolysis of ATP, for which the Cu^{2+}/N-7 interaction is important,[21,25] is inhibited to the extent of the formation of the $Cu(ATP)(Im)^{2-}$ complex.[30]

To conclude, the results described in parts 1 and 2, together with those mentioned from earlier studies,[14,16] indicate that mixed-ligand complex formation between $M(ATP)^{2-}$ and a monodentate or bidentate ligand leads in general to a release of the adenine moiety from the coordination sphere of the metal ion. This release of N-7 is independent of the size and properties of the second ligand; it has now been observed for ligands as different as OH^-, NH_3, imidazole, 2,2′-bipyridyl, and tryptophanate. Clearly, this result affects the expected structures for enzyme/metal ion/ATP complexes.

3. Confirmation of N-7 Release by Stability Constant Analysis of Imidazole Binding to $M(ATP)^{2-}$ Complexes. As described previously,[13-15] metal ions may interact with nucleoside 5′-triphosphates (NTP^{4-}) to give binary complexes that exist in "open" and, for purine nucleotides, also in "closed" forms (eq 1 and 2).

$$M^{2+} + NTP^{4-} \rightleftharpoons M(NTP)^{2-}_{op} \rightleftharpoons M(NTP)^{2-}_{cl} \quad (2a)$$

$$K^{M}_{M(NTP)_{op}} = [M(NTP)^{2-}_{op}]/([M^{2+}][NTP^{4-}]) \quad (2b)$$

$$K_I = [M(NTP)^{2-}_{cl}]/[M(NTP)^{2-}_{op}] \quad (2c)$$

$$\text{overall: } K^{M}_{M(NTP)} = ([M(NTP)^{2-}_{op}] + [M(NTP)^{2-}_{cl}])/([M^{2+}][NTP^{4-}]) = K^{M}_{M(NTP)_{op}}(1 + K_I) \quad (2d)$$

The closed forms consist of a macrochelate in which the metal-ion-bound phosphate also binds to N-7 of a purine base either directly in an inner-sphere complex or indirectly through a water molecule in an outer-sphere complex. In this paper we do not distinguish between these closed or back-bonded forms; their resolution is described elsewhere.[15]

The reaction of an $M(ATP)^{2-}$ complex with another ligand (L) such as OH^-, NH_3, or imidazole (Im) to yield a ternary complex may be described by the equilibria

$$M(NTP)^{2-}_{op} + L \rightleftharpoons (L)M(NTP)^{2-}_{op} \quad (3a)$$

$$K^{M(NTP)}_{M(NTP)(L)_{op}} = [(L)M(NTP)^{2-}_{op}]/([L][M(NTP)^{2-}_{op}]) \quad (3b)$$

The open complex $(L)M(NTP)^{2-}_{op}$ may close with N-7 as does the binary complex:

$$(L)M(NTP)^{2-}_{op} \rightleftharpoons (L)M(NTP)^{2-}_{cl} \quad (4a)$$

$$K_{1/L} = [(L)M(NTP)^{2-}_{cl}]/[(L)M(NTP)^{2-}_{op}] \quad (4b)$$

Alternatively, the nucleic base residue and metal-ion-bound im-

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Table I. Stability Constants of Binary $M(ATP)^{2-}$ and $M(UTP)^{2-}$ Complexes and of the Corresponding Ternary Complexes with Imidazole (I = 0.1 ($NaNO_3$, $NaClO_4$); 25 °C)[a]

M^{2+}	log $K^{M}_{M(ATP)}$	log $K^{M}_{M(UTP)}$	log Δ	log $K^{M(ATP)}_{M(ATP)(Im)}$	log $K^{M(UTP)}_{M(UTP)(Im)}$	log Δ_{Im}	log ($\Delta\Delta_{Im}$)
Mn^{2+}	5.01 ± 0.05	4.91 ± 0.05	0.10 ± 0.07	1.05 ± 0.10	1.27 ± 0.09	−0.22 ± 0.13	−0.12 ± 0.15
Co^{2+}	4.97 ± 0.06	4.73 ± 0.04	0.24 ± 0.07	1.85 ± 0.06	2.04 ± 0.03	−0.19 ± 0.07	0.05 ± 0.10
Ni^{2+}	4.86 ± 0.03	4.47 ± 0.02	0.39 ± 0.04	2.44 ± 0.02	2.63 ± 0.03	−0.19 ± 0.04	0.20 ± 0.06
Cu^{2+}	6.34 ± 0.02	5.87 ± 0.02	0.47 ± 0.03	3.53 ± 0.02	3.84 ± 0.02	−0.31 ± 0.03	0.16 ± 0.04
Zn^{2+}	5.16 ± 0.04	5.01 ± 0.02	0.15 ± 0.04	2.41 ± 0.02	2.54 ± 0.02	−0.13 ± 0.03	0.02 ± 0.05
Cd^{2+}	5.34 ± 0.02	5.10 ± 0.02	0.24 ± 0.03	2.03 ± 0.04	2.33 ± 0.03	−0.30 ± 0.05	−0.06 ± 0.06

[a] The stability constants of the binary $M(NTP)^{2-}$ complexes are from ref 15 (error range 2σ) and those of the ternary $M(NTP)(Im)^{2-}$ complexes from ref 20 (error range 3σ).

idazole in a ternary complex may undergo stacking between the aromatic rings:

$$(L)M(NTP)^{2-}_{op} \rightleftharpoons (L)M(NTP)^{2-}_{st} \quad (5a)$$

$$K_{st} = [(L)M(NTP)^{2-}_{st}]/[(L)M(NTP)^{2-}_{op}] \quad (5b)$$

The stability constant calculated from experimental data for imidazole binding is given by eq 6.

$$K^{M(NTP)}_{M(NTP)(L)} = \frac{[(L)M(NTP)^{2-}_{op}] + [(L)M(NTP)^{2-}_{cl}] + [(L)M(NTP)^{2-}_{st}]}{[L]([M(NTP)^{2-}_{op}] + [M(NTP)^{2-}_{cl}])} = K^{M(NTP)}_{M(NTP)(L)_{op}} \frac{1 + K_{I/L} + K_{st}}{1 + K_I} \quad (6)$$

With $M(UTP)^{2-}$ complexes closed, back-bonded forms do not occur and eq 2 and 6 reduce to $K^{M}_{M(UTP)} = K^{M}_{M(NTP)_{op}}$ and $K^{M(UTP)}_{M(UTP)(L)} = K^{M(NTP)}_{M(NTP)(L)_{op}}(1 + K^{U}_{st})$, respectively, where K^{U}_{st} refers to the equilibrium constant for the formation of the stacked complex for the nucleotide with a uracil base. Thus potentiometric pH titrations with UTP give the stability constant $K^{M}_{M(NTP)_{op}}$ and the product $K^{M(NTP)}_{M(NTP)(L)_{op}}(1 + K^{U}_{st})$.

For the binary complexes of ATP^{4-} and UTP^{4-} we define for the experimentally accessible ratio of the stability constants (see eq 2) eq 7. For the ternary complex with imidazole (eq 6) we

$$\frac{K^{M}_{M(ATP)}}{K^{M}_{M(UTP)}} = \Delta = \frac{K^{M}_{M(ATP)}}{K^{M}_{M(ATP)_{op}}} = 1 + K_I \quad (7)$$

define Δ_{Im} in an analogous way from the experimentally obtainable ratio as given in eq 8. In the last equation a distinction is made

$$\frac{K^{M(ATP)}_{M(ATP)(Im)}}{K^{M(UTP)}_{M(UTP)(Im)}} = \Delta_{Im} = \frac{K^{M(ATP)}_{M(ATP)(Im)}}{K^{M(ATP)}_{M(ATP)(Im)_{op}}} \frac{1}{1 + K^{U}_{st}} = \frac{1 + K_{I/L} + K^{A}_{st}}{(1 + K_I)(1 + K^{U}_{st})} \quad (8)$$

between the equilibrium constants for metal ion bound imidazole stacking, K^{A}_{st} in $M(ATP)(Im)^{2-}$ and K^{U}_{st} in $M(UTP)(Im)^{2-}$. Since back-bonded closed structures can exist only in $M(ATP)^{2-}$, $\Delta \geq 1$. In contrast, imidazole interacts more strongly with the non-back-bonded $M(UTP)^{2-}$ and $\Delta_{Im} \leq 1$. Experimental values for log Δ and log Δ_{Im} appear in the fourth and seventh columns of Table I, respectively.

The importance of base back-binding and stacking to the adenine residue in $M(ATP)(Im)^{2-}$ may be assessed by comparing the observed stability constants with and without imidazole for ATP^{4-} and UTP^{4-}. The combination of eq 7 and 8 yields eq 9.

$$\frac{K^{M}_{M(ATP)}}{K^{M}_{M(UTP)}} \frac{K^{M(ATP)}_{M(ATP)(Im)}}{K^{M(UTP)}_{M(UTP)(Im)}} = \Delta\Delta_{Im} = \frac{1 + K_{I/L} + K^{A}_{st}}{1 + K^{U}_{st}} \quad (9)$$

The left-hand side of eq 9 contains only experimentally accessible stability constants.[15,20] This means that the values of log Δ in the fourth column of Table I may be combined with the log Δ_{Im} values in the seventh column to obtain the log ($\Delta\Delta_{Im}$) values tabulated in the last column of Table I.

Inspection of the last column in Table I reveals that log ($\Delta\Delta_{Im}$) $\simeq$ 0 within experimental error for Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+}. To obtain log ($\Delta\Delta_{Im}$) = 0 or $\Delta\Delta_{Im}$ = 1, eq 9 indicates that both the back-bonding in the $M(ATP)(Im)^{2-}$ complexes must be negligible, $K_{I/L} \simeq 0$, and the imidazole stacking interaction must be of the same extent in $M(ATP)(Im)^{2-}$ and $M(UTP)(Im)^{2-}$; i.e., $1 + K^{A}_{st} = 1 + K^{U}_{st}$.[31] The conclusion that $K_{I/L} \simeq 0$ agrees completely with the ^1H NMR shift evidence presented in part 2 for the $Zn^{2+}/ATP^{4-}/Im$ and $Cd^{2+}/ATP^{4-}/Im$ systems. We conclude that upon binding of imidazole all back-bonded N-7 of the adenine residue (Figure 1) is released in the ternary $M(ATP)(Im)^{2-}$ complexes of Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+}.

For Ni^{2+} and Cu^{2+} the values of log ($\Delta\Delta_{Im}$) are different from zero beyond the experimental uncertainty (Table I, last column); i.e., $\Delta\Delta_{Im}$ = 1.6 and 1.4, respectively. Equation 9 indicates that to obtain values of $\Delta\Delta_{Im} > 1$, $K_{I/L} > 0$ and/or $K^{A}_{st} > K^{U}_{st}$. Hence, we are left with two limiting explanations for log ($\Delta\Delta_{Im}$) > 0 in the case of Ni^{2+} and Cu^{2+}. However, we still may conclude that N-7 back-binding in $Ni(ATP)(Im)^{2-}$ and $Cu(ATP)(Im)^{2-}$ must be reduced in comparison with that in $Ni(ATP)^{2-}$ and $Cu(ATP)^{2-}$, respectively, because log Δ > log ($\Delta\Delta_{Im}$) (see Table I), if also $K^{A}_{st} > K^{U}_{st}$ remains unknown.

4. Further Evidence for N-7 Release by Stability Constant Comparisons of $M(NTP)(OH)^{3-}$ Complexes. A similar analysis as given in part 3 is possible for ternary hydroxo complexes. However, from the known[14,28] stability data only the results for $Zn(ATP)(OH)^{3-}$ and $Zn(CTP)(OH)^{3-}$ (CTP^{4-} = cytidine 5′-triphosphate) are of sufficient precision[28] to permit an assessment of the effect of the hydroxo coordination on N-7 back-bonding in $Zn(ATP)^{2-}$. The hydroxo complex of $Zn(CTP)^{2-}$ may be used as a basis here, because in $Zn(CTP)^{2-}$ no Zn^{2+}/cytosine moiety interaction occurs.[13]

A comprehensive collection of stability constants tabulates[15] log $K^{Zn}_{Zn(ATP)} = 5.16 \pm 0.04$ and log $K^{Zn}_{Zn(CTP)} = 5.03 \pm 0.05$, from which log $\Delta = 0.13 \pm 0.06$ (2σ) in analogy to eq 7. Upon pH titration of the complexes a hydroxo ion binds to Zn^{2+} with $pK^{H}_{Zn(ATP)(H_2O)} = 8.87 \pm 0.04$ and $pK^{H}_{Zn(CTP)(H_2O)} = 8.79 \pm 0.05$.[28] Analogously to imidazole binding and the definition of Δ_{Im} in eq 8 we obtain for hydroxide binding log $\Delta_{OH} = -0.08 \pm 0.06$ (3σ). Addition according to eq 9 yields log ($\Delta\Delta_{OH}$) = 0.05 ± 0.08. Thus within the experimental uncertainty hydroxide binding to Zn-

(31) As the limiting size of the aromatic residues is given by the size of the imidazole ring, a comparable extent of stack formation in $M(ATP)(Im)^{2-}$ and $M(UTP)(Im)^{2-}$ is quite possible. That the uridine residue can participate in stacking interactions in ternary complexes is well-known.[11,16] Indeed, a ^1H NMR shift experiment in D_2O with [Zn^{2+}] = [UTP] = 0.03 M at pD 8.0 and 34 °C on a Varian Anaspect EM-360 spectrometer (60 MHz) using the center peak of the tetramethylammonium ion triplet as internal reference gave upon addition of imidazole (0.07 M) the following upfield shifts: $\Delta\delta$ = 0.037 for H-6, 0.097 for H-5, and 0.096 for H-1′. Repetition of the experiment under the same conditions but in the absence of Zn^{2+} gave $\Delta\delta$ = 0.011 for H-6, 0.014 for H-5, and 0.010 for H-1′. This indicates (cf. Figures 3 and 4) that formation of an intramolecular metal ion bridge facilitates stacking between the imidazole ring and the uracil moiety; under the above conditions the ternary $Zn(UTP)(Im)^{2-}$ complex is formed to about 85% (based on [Zn^{2+}] = 0.03 M). It should be added that addition of Zn^{2+} to UTP (1:1) under the above conditions also induces slight upfield shifts due to the known[13] metal-ion-promoted self-association; these upfield shifts are 0.060 ppm for H-6, 0.009 ppm for H-5, and 0.005 ppm for H-1′.

Table II. Extent of Intramolecular Macrochelate Formation in $M(ATP)^{2-}$ Complexes from a Comparison of the Stability Constants of Corresponding Ternary $M(ATP)(Im/OH)^{2-/3-}$ and $M(UTP/CTP)(Im/OH)^{2-/3-}$ Complexes (I = 0.1 ($NaNO_3$); 25 °C)[a]

M^{2+}	log Δ_L (eq 10)	K_I (eq 11)	% $M(ATP)^{2-}_{cl}$ (eq 1)	% $M(ATP)^{2-}_{cl}$ (ref 15)
Mn^{2+}	−0.22 ± 0.13[b]	0.66 ± 0.51[d]	40 ± 19[d]	17 ± 10
Co^{2+}	−0.19 ± 0.07[b]	0.55 ± 0.24	35 ± 10	38 ± 9
Ni^{2+}	−0.19 ± 0.04[b]	>0.55 ± 0.13[e]	>35 (±5)[e]	56 ± 4
Cu^{2+}	−0.31 ± 0.03[b]	>1.04 ± 0.13[e]	>51 (±3)[e]	67 ± 2
Zn^{2+}	−0.13 ± 0.03[b]	0.35 ± 0.09	26 ± 5	28 ± 7
	−0.08 ± 0.06[c]	0.20 ± 0.18	17 ± 12	
Cd^{2+}	−0.30 ± 0.05[b]	1.00 ± 0.23	50 ± 6	46 ± 4

[a] For the reasoning behind this evaluation see text in part 5. The error given with the constants (and otherwise) is 3 times the standard error of the mean value. [b] $\log \Delta_{Im} = \log K^{M(ATP)}_{M(ATP)(Im)} - \log K^{M(UTP)}_{M(UTP)(Im)}$; see Table I. [c] Based on $Zn(ATP)(OH)^{3-}$ and $Zn(CTP)(OH)^{3-}$; see text in part 4 regarding $\log \Delta_{OH}$. [d] For $Mn(ATP)^{2-}$ an evaluation is actually not justified due to the low stability of the related ternary complexes, which leads to rather large error limits of the available constants for $Mn(ATP)(Im)^{2-}$ and $Mn(UTP)(Im)^{2-}$, though the calculated result is in fact still quite reasonable. [e] These values are lower limits; see text in part 5.

$(ATP)^{2-}$ results in release of all back-bonded N-7 groups in $Zn(ATP)(OH)^{3-}$.

This conclusion derived from stability constants is in complete agreement with those obtained by ^1H NMR spectroscopy.[14] The latter method indicates that binding of hydroxide also completely releases back-bonded N-7 not only in $Zn(ATP)(OH)^{3-}$ but also in $Cd(ATP)(OH)^{3-}$. Similar ^1H NMR experiments (see part 1) reveal that NH_3 also releases inner-sphere N-7 in $Cd(ATP)^{2-}$ upon formation of $Cd(ATP)(NH_3)^{2-}$. Kinetic experiments on the metal-ion-facilitated dephosphorylation of ATP also indicate the release of N-7 from the coordination sphere of the metal ion upon formation of $Cu(ATP)(OH)^{3-}$, $Zn(ATP)(OH)^{3-}$, and $Cd(ATP)(OH)^{3-}$;[21] the formation of the hydroxo complexes is connected with a decreasing reaction rate.

5. Estimates for the Extent of the Macrochelated Form of $M(ATP)^{2-}$ from the Stabilities of Ternary Complexes. Analysis of the stability data for the complexes with Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} according to eq 9 revealed that $K_{I/L} \simeq 0$ for the ternary $M(ATP)(Im)^{2-}$ complexes (part 3) and for $Zn(ATP)(OH)^{3-}$ as well (part 4). In addition, the same analysis showed also that $K^A_{st} \simeq K^U_{st}$ for the $M(ATP)(Im)^{2-}$ complexes (part 3). This observation allows connection of the stability constants, $K^{M(NTP)}_{M(NTP)(L)}$, of the corresponding ternary $M(NTP)(Im)^{2-}$ and $Zn(NTP)(OH)^{3-}$ complexes with the extent of base back-binding in the binary $M(ATP)^{2-}$ species. This means that eq 8 reduces for the given systems and the *aforementioned conditions* to eq 10.

$$\frac{K^{M(ATP)}_{M(ATP)(Im)}}{K^{M(UTP)}_{M(UTP)(Im)}} = \Delta_{Im} = \frac{1}{1 + K_I} \qquad (10a)$$

$$\frac{K^{H}_{Zn(ATP)(OH)}}{K^{H}_{Zn(CTP)(OH)}} = \Delta_{OH} = \frac{1}{1 + K_I} \qquad (10b)$$

Equation 10 may be rewritten as eq 11.

$$K_I = \frac{[M(ATP)^{2-}_{cl}]}{[M(ATP)^{2-}_{op}]} = 10^{-\log \Delta_L} - 1 \qquad (11)$$

The results based on eq 11 are summarized in Table II for the $M(ATP)^{2-}$ complexes with Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+}. The imidazole and OH^- values calculated for $Zn(ATP)^{2-}_{cl}$ agree with each other. The value given for $Mn(ATP)^{2-}_{cl}$ is only a very rough estimate due to the rather large error limit, but despite this shortcoming the observed value for $\log \Delta_{Im}$ still confirms the formation of macrochelates in this system.

For the Ni^{2+} and Cu^{2+} systems $\log (\Delta\Delta_{Im}) > 0$ (Table I) as discussed in part 3. This means in eq 8 $(1 + K_{I/L} + K^A_{st})/(1 + K^U_{st}) > 1$, and therefore, eq 11 provides only *lower* limits for the extent of base back-binding in $Ni(ATP)^{2-}$ and $Cu(ATP)^{2-}$; these lower limits are also listed in Table II.

Overall, the percentages calculated for $M(ATP)^{2-}_{cl}$ via the stability constants of ternary complexes agree well with a comprehensive evaluation of stability data of binary complexes (see last column in Table II). Indeed, the main gain of the present evaluation is the confirmation by an independent experimental method of the extent of the macrochelate formation in several $M(ATP)^{2-}$ complexes.

Conclusions

Addition of the unidentate ligands OH^-, NH_3, and imidazole completely eliminates N-7 back-bonding in the binary $M(ATP)^{2-}$ complexes of Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+}. Addition of imidazole at least weakens and possibly eliminates N-7 back-bonding in $Ni(ATP)^{2-}$ and $Cu(ATP)^{2-}$. These results suggest that when bound to an enzyme the binary complexes may exist in a closed macrochelate form only if no enzyme groups coordinate directly to the metal ion.[32]

Furthermore, as $M(ATP)^{2-}$ complexes are the actual substrates for enzymes and not uncomplexed ATP^{4-}, the evaluated isomeric equilibria as well as the structural alterations occurring upon mixed-ligand complex formation may play a role in the enzymic selectivity. For example, Mn^{2+} is an activator for many nucleotide/enzyme systems[6] and RNA polymerases[33] are Zn^{2+} metalloenzymes.

Acknowledgment. We thank K. Aegerter of the Institute of Organic Chemistry for recording the 90-MHz ^1H NMR spectra. Part of the calculations were done with computers made available by the Rechenzentrum der Universität Basel (Univac 1100/81). This support and research grants from the Swiss National Science Foundation (H.S.) are gratefully acknowledged.

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