Contribution from the Institute of Inorganic and Analytical Chemistry, Lajos Kossuth University, H-4010 Debrecen, Hungary, and Chemistry Department, University of Virginia, Charlottesville, Virginia 22903

Al^{3+} Binding by Adenosine 5'-Phosphates: AMP, ADP, and ATP

Tamás Kiss,*,† Imre Sóvágó,*,† and R. Bruce Martin*,‡

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To assess quantitatively the ability of Al^{3+} to complete with Mg^{2+} in many vital processes, we have determined the stability constants for Al^{3+} binding to adenosine 5'-phosphates. With ATP, Al^{3+} forms an equimolar complex 4000 times stronger than does Mg^{2+}, and stronger than most metal ions including Cu^{2+}. In addition to an equimolar complex, Al^{3+} also forms a complex with 2 mol of ligand that dominates in slightly acidic and neutral solutions, from which a deprotonation occurs to form a mixed hydroxo complex. ADP forms only slightly weaker complexes than ATP. Because Al^{3+} binding occurs primarily at the basic, terminal phosphate, the results also apply to other nucleotides such as GTP. Binding by 10 mM nucleoside triphosphate at pH 6.6 limits the free Al^{3+} to 3×10^{-9} times the total aluminum concentration. ATP stands as the predominant binder of Al^{3+} in many cells. In neutral solutions, citrate withdraws Al^{3+} from ATP. In a low citrate medium, catecholamines extract Al^{3+} from ATP. The $10^{7.5}$ times greater effectiveness of Al^{3+} over Mg^{2+} in promoting tubulin polymerization to microtubules derives from the $10^{3.6}$ times greater binding strength of Al^{3+} over Mg^{2+} to GTP coupled with the $10^{3.9}$ times greater binding strength of GTP-Al^{3+} over GTP-Mg^{2+} to tubulin.

Concern over the appearance of increasing Al^{3+} concentrations in acidified natural waters has deepened since demonstration of its association with several pathological states in humans, including bone disorders and dementias such as Alzheimer's disease. The mode of Al^{3+} action remains uncertain, but a recurring theme is its association with phosphate groups. Because some aluminum locates in the nuclei of neurons in neurofibrillary tangles, many suggest Al^{3+} binding to DNA. However, the negatively charged phosphate groups on DNA are not basic ($pK_a \sim 1$) and unless there are unusual structural features, DNA does not bear specific binding sites for a metal ion such as Al^{3+}, which interacts merely as a counterion to a polyelectrolyte. More likely, Al^{3+} binds to more basic phosphate groups that occur in phosphorylated proteins and nucleoside phosphates.

Similar in size to the natural activator Mg^{2+}, Al^{3+} may act by substituting for Mg^{2+} in vital processes.[1,2] For example, in tubulin polymerization to microtubules, Al^{3+} is more than 10^7 times as effective as Mg^{2+}.[3] Mg^{2+} serves as a natural activator by binding to the phosphates of guanosine triphosphate (GTP), and Al^{3+} displaces Mg^{2+} at this site. Virtually all ATP-associated reactions use Mg^{2+}, and Al^{3+} potentially interferes in these processes. However, reliable stability constants for Al^{3+} binding to nucleoside phosphates are unknown, and remedying this deficiency stands as an important need in Al^{3+} biochemistry. This article reports stability constants for Al^{3+} binding to adenosine 5'-phosphates and, by extension to other nucleoside phosphates, applies the results to the interpretation of systems utilizing nucleoside phosphates.

Only two studies have reported stability constants for Al^{3+} binding to ATP, and both bear deficiencies. A kinetic procedure utilizing yeast hexokinase gave an apparent stability constant of $\log K = 6.2$ at pH 6.95.[4] To obtain the normal or usual stability constant, this value needs to be increased to allow for the extensive hydrolysis of Al^{3+} in neutral solutions.[1,5] On the basis of the more direct results reported in this paper, the indirect kinetic approach appears faulty. A more recent study reports much stronger binding of Al^{3+} to ADP than to ATP and describes "unusual" formation curves that "are not easily interpreted".[6] For these reasons and because of the fact that other metal ions bind more strongly to ATP than to ADP,[7,8] this study does not appear reliable. Neither of these two studies describes 2:1 complexes, essential for assessing the free Al^{3+} concentration in many systems.

Experimental Section

Adenosine 5'-phosphates were the best quality sodium salts available from Sigma Chemical Co., St. Louis, MO. Potentiometric titrations were performed with a Radiometer PHM64 pH meter and TTA 80 automatic titration unit equipped with G2040B glass and K4040 calomel electrodes. Ligand concentrations were 4 and 8 mM, and the ligand to Al^{3+} mole ratios were 1, 2, 4, 7, 8, and 10 to 1. Titration curves with equimolar amounts of Al^{3+} and ligand may be treated only to pH ~3.5 for AMP and ADP and to pH ~4.5 for ATP. With excess ligand, the safe range extends to pH ~4 for AMP and pH ~8 for ADP and ATP. Within these regions, constancy of the pH meter reading could be achieved in a reasonable time (~2-5 min), and the fit between experimental (30-40 points) and calculated (PSEQUAD)[9] titration curves was excellent. For the hydroxo complexes of Al^{3+}, the stability constants ($\log \beta$) assumed[10] were -5.52 for $Al(OH)^{2+}$ and -23.46 for $Al(OH)_4^-$; polynuclear complexes are not significant in the presence of ligand. Experiments were performed at 25 °C with the ionic strength controlled at 0.2 M with KCl. The $\log \beta$ values reported are concentration constants.

Table I. Adenosine 5'-Phosphate Acidity Constants and Their Al^{3+} Complex Stability Constants[a]

species	AMP	ADP	ATP
HA	6.04 (1)	6.19 (1)	6.31 (1)
H_2A	9.78 (2)	9.98 (2)	10.20 (2)
AlAH		10.98 (4)	11.30 (4)
AlA	6.17 (1)	7.82 (3)	7.92 (4)
$AlAH_{-1}$	2.02 (9)	2.94 (8)	2.46 (7)
AlA_2	10.35 (11)	12.16 (4)	12.47 (4)
AlA_2H_{-1}	*b*	5.01 (7)	4.84 (5)
pK_A	3.74	3.79	3.89
pK_P	6.04	6.19	6.31
pK_{MAH}		3.16	3.38
$\log K_1$	6.17	7.82	7.92
pK_{MA}	4.15	4.88	5.46
$\log K_2$	4.18	4.34	4.55
pK_{MA2}		7.15	7.63

[a] As logarithms at 25 °C and 0.2 M ionic strength controlled with KCl. Acidity constants are concentration constants. Upper half of table contains $\log \beta$ values, and lower half constants are defined in the text. [b] Precipitation.

Results

Table I lists the results, in terms of the objective species analysis in the upper half and in terms of the more conventional constants

† Lajos Kossuth Univeristy.
‡ University of Virginia.

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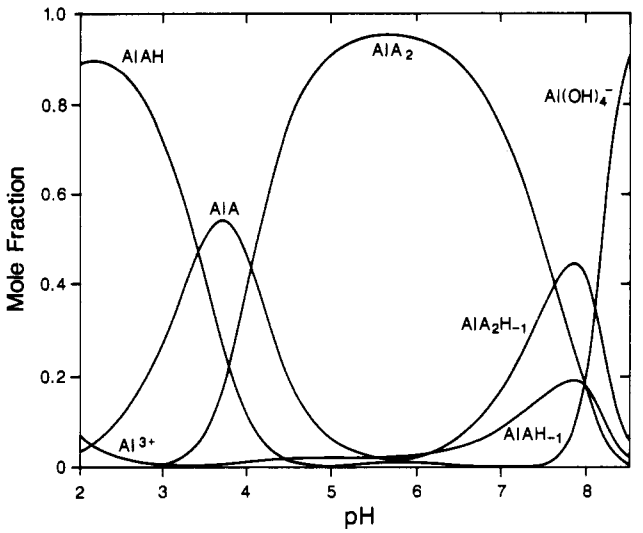

Figure 1. Plot of mole fraction, Al^{3+} basis, versus pH for a solution containing 0.1 mM total aluminum and 10 mM total 5'-ATP using the constants in Table I. The disposition of the curves is virtually independent of the total aluminum concentration and not highly dependent on the ATP concentration. At less than 4 mM ATP, the concentration of $AlAH_{-1}$ surpasses that of Al_2H_{-1}. The A refers to tetraanionic ATP^{4-}.

in the lower half. The symbol A designates the 2- species for AMP, the 3- species for ADP, and the 4- species for ATP. Numbers in parentheses represent one standard deviation in the last digit quoted.

The successive acidity constants pK_A (~3.8) and pK_P (~6.2) represent deprotonations from N1 of the adenine base and from the terminal phosphate group, respectively. The values in Table I are concentration constants 0.10–0.17 log unit lower than widely used activity-based values for AMP[11] and ATP.[12] In addition to the activity coefficient of H^+, the difference between the two sets of values is due mainly to differing background electrolytes and their concentrations, 0.2 M KCl and 0.1 M $NaNO_3$.[13]

With pK_P = 6.04–6.31 in Table I, the basic, terminal phosphate group becomes the primary site for Al^{3+} binding. The stability constant for formation of the equimolar complex AlA (log K_1) is greater than that for most metal ions including Cu^{2+}.[12] In addition, Al^{3+} forms strong 2:1 complexes with a second mole of ligand. The comparable constants in Table I indicate that ADP complexes are only slightly weaker than those of ATP.

Figure 1 shows the species distribution for ATP presented as mole fraction of aluminum versus pH. Beginning in acid solution, the first complex species, AlAH, loses a proton from N1 of the adenine base with pK_{MAH} = 3.4 to give AlA. Owing to the presence of Al^{3+} on the phosphates, the acidity at N1 is promoted compared to the case of the free ligand (pK_A = 3.9). Beginning about pH 4, the 2:1 complex AlA_2 dominates until pH 7, when the mixed hydroxo complexes AlA_2H_{-1} ((HO)AlA_2) and $AlAH_{-1}$ ((HO)AlA) become important. Finally, at pH >8, the ATP complexes become unstable with respect to $Al(OH)_4^-$. The difference between values for the AlAH and HA species in the third and first rows of Table I refers to transfer of the phosphate proton to N1 and complexation of Al^{3+} at the phosphate.

Discussion

The first stability constant, log K_1, for Al^{3+} binding to the completely deprotonated phosphate groups increases only modestly through the series AMP → ADP → ATP as 6.17 → 7.82 → 7.92. To allow for the accompanying small increase in phosphate group basicity, it is more appropriate to compare the difference log K_1 – pK_P, which is 0.13 → 1.63 → 1.61 through the series. This comparison indicates only a 1.5 log unit gain owing to chelation by addition of a second phosphate group in ADP and no gain for addition of the third in ATP. This result suggests Al^{3+} coordination to the two terminal phosphate groups in ADP and ATP, with no coordination to the α-phosphate group in ATP. This conclusion agrees with the analysis presented for a variety of metal ions.[8]

The weakness of Al^{3+} interaction with a second phosphate group is supported by the results for binding of a second ligand. For the series AMP → ADP → ATP, the log K_2 values are 4.18 → 4.34 → 4.55. When adjusted for increasing phosphate group basicity, the difference log K_2 – pK_P becomes −1.86 → −1.85 → −1.76. The near constancy of these values suggests that only the terminal phosphate of a second ligand binds in forming AlA_2.

Though only a single phosphate group coordinates to Al^{3+} in AMP and in the second ligand for ADP, and ATP, one must be cautious in describing the denticity of the ligand and the coordination number of Al^{3+}. Chelation of Al^{3+} by an α-phosphate oxygen and an oxygen on the ribose ring is sterically unfavorable and unlikely to contribute significantly to stability. Even when a metal ion is bound to only one phosphate, the group may still be bidentate through two oxygen atoms in a four-membered chelate ring. That the coordination sphere of aqueous Al^{3+} is reduced from hexacoordinate to a lesser number is suggested by the low pK_{MA} values in Table I, due to formation of a mixed hydroxo complex, which are slightly more acidic than the value for the first deprotonation from aqueous Al^{3+}. Instead of a typical gap of at least 1 log unit between successive deprotonations, for aqueous Al^{3+} four deprotonations from $Al(H_2O)_6^{3+}$ to tetrahedral $Al(OH)_4^-$ occur over a short span of less than 1 log unit. This cooperative process has been attributed to the reduction in coordination number.[1] Unusually acidic pK_a values occur for other complexes and in all cases may be ascribed to a concomitant reduction in coordination number.[1]

Despite the presence of two basic phosphate groups (pK_a > 5.9) in 2,3-diphosphoglycerate, it binds Al^{3+} in a weaker fashion than either ADP or ATP and with stability constants comparable to those for AMP.[14] (All three nucleoside phosphates bear only one (the terminal) basic phosphate group.) These comparisons support the conclusion that only the 2-phosphate group complexes Al^{3+} in 2,3-diphosphoglycerate.[14]

Like Mg^{2+}, Ca^{2+},[12] and the lanthanides,[15] Al^{3+} binds to all nucleoside phosphates predominantly at the phosphate groups. The only basic phosphate group (pK_a > 6) is the terminal one. Since the nucleoside triphosphates exhibit similar phosphate pK_a values, as do the nucleoside diphosphates and nucleoside monophosphates, the equilibrium constants in Table I apply to other nucleoside phosphates such as GTP and GDP.

On the basis of an upfield H8 shift in ^1H NMR spectroscopy, it has been proposed that Al^{3+} binds to N7 of the adenine base in ATP.[16] However, diamagnetic metal ion binding at N7 produces downfield shifts at H8.[17] An upfield H8 shift is due to nucleic base deprotonation at N1 or base stacking.[18] None of the four structures proposed in the NMR study correspond to species described in this article.

Although the free nucleoside phosphate ligands are minimally stacked under the conditions of this study, the charge neutralization provided by binding of Al^{3+} should promote base stacking. Stacking between two ligands bound to a single Al^{3+} may abet formation of 2:1 complexes. The extent of stacking in Al^{3+} complexes of nucleoside phosphate remains to be determined by NMR spectroscopy along the lines employed for other diamagnetic metal ions.[18]

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The free Al^{3+} molar concentration permitted by different ligands under a variety of conditions is a useful quantity for comparison purposes. Figure 1 shows that at pH >4 the 2:1 ATP complex becomes the dominant species. (A similar condition also applies to ADP.) Under conditions of a large excess of a strong binding ligand in the AlA_2 complex region, we may approximate the free metal ion concentration by $[Al^{3+}] \sim C_M/(K_1K_2C_A^2f)$, where C_M and C_A are the total metal ion and ligand concentrations, respectively. Also included is a factor, $f = 1 + K_{MA2}/(H^+)$ that allows for deprotonation of the AlA_2 complex to AlA_2H_{-1}. At $pH < pK_{MA2}$, f approaches unity; at $pH = pK_{MA2}$, $f = 2$. On an activity scale for H^+, we have $pK_{MA2} = 7.3$ for ADP and 7.8 for ATP. For 10 mM ATP, typical of the inside of a cell, the free Al^{3+} concentration becomes $[Al^{3+}] = C_M/(10^{12.5} \times 10^{-4}f)$. At an intracellular pH of 6.6, we obtain $f = 1.06$, and for a pH of 7.4, $f = 1.4$. Therefore at pH 6.6, $[Al^{3+}] = 3 \times 10^{-9}C_M$ so that for 1 μM total aluminum the free Al^{3+} concentration $[Al^{3+}] = 3 \times 10^{-15}$ M. Thus, owing to formation of a 2:1 complex, ATP markedly reduces the free Al^{3+} concentration. The level is 100 times less than that allowed by 10 mM phosphate within a cell.[1,5] Thus, ATP stands as the predominant binder of Al^{3+} in many cells.

In a cell, however, Al^{3+} competes with Mg^{2+} for a binding site on ATP. For Mg^{2+} and ATP, the stability constant log $K_1 = 4.3$,[12] 3.6 log units or a factor of 4000 less than that of Al^{3+} in Table I. Taking intracellular Mg^{2+} as 1 mM, we find that Al^{3+} competes with Mg^{2+} at a level of only 0.1 μM. Any ATP not saturated with Mg^{2+} will complex even lower levels of Al^{3+}.

Depending upon their concentrations, other ligands may compete with ATP for Al^{3+}. Citrate is a strong Al^{3+} binder in neutral solutions[1,19] and at comparable or lower concentrations withdraws Al^{3+} from ATP. At the intercellular pH 6.6, 0.1 mM citrate[1] binds Al^{3+} as strongly as 6 mM ATP, a 60 times greater concentration. Both ATP and the catecholamines form 2:1 complexes. The successive conditional stability constants applicable to pH 7 for L-Dopa, dopamine, norepinephrine, and epinephrine yield average values of log $K_{1C7} = 8.2$ and log $K_{2C7} = 5.5$.[21] These values are greater than the ATP values from Table I log $K_1 = 7.9$ and log $K_2 = 4.6$. Thus, at comparable ligand concentrations in neutral solutions, the catecholamines bind Al^{3+} more strongly than ATP. In fluids low in citrate concentration, such as the cerebrospinal fluid, the inability of ATP to withdraw Al^{3+} from catecholamines may be deleterious.

The literature already reports important interactions involving Al^{3+} and nucleoside triphosphates.[4,20,22] For years it had been known that certain metabolites such as phosphate and citrate activate yeast and brain hexokinase enzymes at pH ≤7. The metabolites thus became implicated in regulating hexokinase function. However, it was discovered that the loss of hexokinase activity at pH ≤7 is due to Al^{3+} contamination of commerical preparations of ATP.[20] Phosphate and citrate "activate" either by forming noninhibitory ternary complexes or by complexing Al^{3+} and freeing the ATP. The variable Al^{3+} contamination is low, of the order of only 1 mol%, and Al^{3+} is the most common metal ion contaminant of ATP preparations.[20,23,24] Inhibition by a contaminant present at just 1% is observed only because the inactive ATP-Al^{3+} complex binds to hexokinase about 700 times more strongly than ATP-Mg^{2+}. Does this enormous hexokinase binding advantage in favor of ATP-Al^{3+} apply to other nucleoside phosphate systems?

Al^{3+} is more than 10^7 times as effective as Mg^{2+} in promoting the assembly of tubulin in microtubules.[3] Mg^{2+} and Al^{3+} bind with guanosine triphosphate (GTP) at receptor sites on tubulin. Experiment showed that Al^{3+} binds to the tubulin–GTP complex $10^{7.5}$ times more strongly than Mg^{2+}. Most of this rate enhancement was attributed to a correspondingly stronger Al^{3+} over Mg^{2+} binding to GTP, but this conclusion depended upon an assumed Al^{3+}-GTP stability constant 3.0 log units greater than that found for ATP in this research. Since Al^{3+} binds primarily to the phosphates, we use the new ATP stability constants of this work for GTP binding and reanalyze the partitioning of the large rate enhancement found in tubulin polymerization. With log $K_1 = 7.9$ for Al^{3+} and log $K_1 = 4.3$ for Mg^{2+}, only 3.6 log units may be assigned to stronger Al^{3+} binding to GTP. The remaining difference of 7.5 − 3.6 = 3.9 log units must be attributed to the stronger binding of the GTP-Al^{3+} complex to tubulin (log K_{MP} in ref 3). Only this partitioning change in the tubulin polymerization interpretation is required by the new stability constants of this paper. This partitioning reanalysis of the tubulin polymerization results reveals a parallel with the hexokinase reaction. In both cases, an appreciable contribution to the effectiveness of Al^{3+} lies not only in its 3.6 log unit stronger binding that of than Mg^{2+} to ATP or GTP but also in a stronger binding of the nucleoside triphosphate–Al^{3+} complex to the macromolecule, 2.9 log units for hexokinase and 3.9 log units for tubulin.

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