

Aluminum complexation with nucleoside di- and triphosphates and implication in nucleoside binding proteins

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Abstract

Aluminum ion (Al^{3+}) is known to affect markedly a wide variety of biological systems, and the presence of Al^{3+} in humans has been linked to a number of diseases. As the role of Al^{3+} in human disease clearly has its origins in Al^{3+} coordination chemistry, and since ATP may well serve as the predominant binder of Al^{3+} in many cells, it is important to understand speciation in systems containing Al^{3+} ion and nucleoside di- and triphosphates (especially ADP and ATP). There are also a large number of proteins which require nucleoside phosphates as substrates or are regulated by nucleoside phosphates, and Al^{3+} ion can potentially interfere with the normal functions of these proteins. The objective of this review is to present the current state of our understanding of aluminum complexation with nucleoside di- and triphosphates and to indicate possible consequences of Al^{3+} complexation with proteins. Much of the current knowledge has been revealed by comprehensive potentiometric titration and multinuclear nuclear magnetic resonance analyses. Computer calculations have also played an important role in expanding our understanding of Al^{3+} complexation with nucleoside phosphates.

Keywords: Aluminium; ADP; ATP; NMR; Potentiometric titrations; Proteins

1. Introduction

Al^{3+} is now known to affect markedly a wide variety of biological systems [1–3]. This has been demonstrated both *in vivo* and *in vitro* [4–6]. Al^{3+} is the likely cause of a number of human diseases, including vitamin-D-resistant osteomalacia, iron adequate microcytic anemia, and dialysis dementia [7–9]. Al^{3+} has also been linked to amyotrophic lateral sclerosis [10,11] and Alzheimer's disease [12–14], although considerable controversy surrounds the latter linkage to Al^{3+} . While uncertainties exist with regard to the relevance of Al^{3+} to certain human disorders, there is little doubt that Al^{3+} is a neurotoxic agent [9] and thus worthy of additional study in this context.

The role of Al^{3+} in human disease clearly has its origins in Al^{3+} coordination chemistry. Studies into the linkage of Al^{3+} chelation and human disorders have proliferated with the realization that environmental Al^{3+} availability has been greatly increased as a consequence of acid rain [9]. The chemistry of Al^{3+} indicates a strong preference for binding to negatively charged oxygen atoms. This is physiologically relevant, as Martin [9] has pointed out that the phosphate oxygen atoms of ATP serve as the predominant binder of Al^{3+} in many cells. In neutral solution (such as we find in blood plasma) Al^{3+} is likely transferred from ATP to citrate.

Considering the large number of proteins which require nucleoside phosphates as substrates or are regulated by nucleoside phosphates, much interest has been stimulated by the potential perturbation of function in nucleoside-binding proteins by Al^{3+} . (Note that it is possible to have either Al^{3+} -induced enhancement or Al^{3+} -induced inhibition of normal protein function. For example, mitochondrial F_1 -ATPase is inhibited by Al^{3+} (in the presence of fluoride ion) [15], while G proteins are generally activated by Al^{3+} (also in the presence of fluoride ion) [16,17].) This is clearly related to the fact that virtually all nucleoside phosphate reactions use Mg^{2+} , and Al^{3+} is known to be an effective surrogate for Mg^{2+} [9]. The perturbation of function, then, must be linked to differences in binding affinity of Al^{3+} and Mg^{2+} for the relevant nucleoside phosphate and/or to differences in the nature of the coordination complex formed with Al^{3+} and Mg^{2+} . There is thus a great need for refining procedures for ascertaining the nature of complexes that exist under any given set of conditions (i.e. concentration of nucleoside phosphate, concentration of Al^{3+} , pH, ionic strength etc.) and for having reliable measures of stability constants for their formation. (Such comprehensive studies have already been performed on nucleoside di- and triphosphate complexes with metals other than Al^{3+} . For example, Scheller and Sigel [18] have examined, by proton nuclear magnetic resonance (NMR), the complexation of both purine and pyrimidine nucleoside diphosphates with Mg^{2+} , Cd^{2+} and Zn^{2+} , while Sigel et al. [19], using the potentiometric titration approach, have evaluated stability constants associated with purine and pyrimidine nucleoside triphosphate complexation with Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cd^{2+} , Zn^{2+} and Cu^{2+} .) In the light of the recognized importance of Al^{3+} -induced perturbation of processes requiring nucleoside phosphates, it is somewhat surprising that there are relatively few stability constant studies, and some of those studies which are extant exhibit significant shortcomings. Early work by Viola et al. [20] used a

kinetic approach to determine (indirectly in a yeast hexokinase system) the stability constant for Al^{3+} binding to ATP. Martin [6,21] indicated that the value reported by Viola et al. ($\log K = 6.2$ at pH 6.95) was significantly underestimated and needed to be increased to allow for Al^{3+} hydrolysis in the neutral solutions employed in the previous study. Later Jackson and Voyi [22,23] reported the unexpected result that Al^{3+} binds more strongly to ADP than to ATP. As this result was contrary to those results obtained in studies of nucleoside complexation to other metals [24], it was challenged in a more comprehensive potentiometric study by Kiss et al. [25], which sought to determine more accurate values for complex stability constants for AMP, ADP and ATP. The study by Kiss et al. indicated that the first stability constant (i.e. $\log K_1$) for Al^{3+} binding to completely deprotonated phosphate groups increases slightly through the series $\text{AMP} \rightarrow \text{ADP} \rightarrow \text{ATP}$ ($6.17 \rightarrow 7.82 \rightarrow 7.92$). Analysis of these values indicated that Al^{3+} coordination (in ADP and ATP) occurs almost exclusively to the two terminal phosphate groups (i.e. the α -phosphate of ATP is not involved), a result consistent with that observed with metals other than Al^{3+} [24]. Table 1, taken from Kiss et al. [25] presents the complete set of adenosine 5'-phosphate acidity constants and Al^{3+} complex stability constants, while Fig. 1, also from Kiss et al., presents a plot of mole fraction, Al^{3+} basis, vs. pH for a solution containing 0.1 mM total aluminum and 10 mM total 5'-ATP using the constants in Table 1. (Since Al^{3+} binds exclusively at the phosphate groups, like Mg^{2+} , Ca^{2+} and the lanthanides, the equilibrium constants in Table 1 and the plots in Fig. 1 are expected to apply equally well to GDP and GTP.)

Table 1

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 ₂ A	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)
(OH)AlA	2.02(9)	2.94(8)	2.46(7)
AlA ₂	10.35(11)	12.16(4)	12.47(4)
(OH)AlA ₂	— ^b	5.01(7)	4.84(5)
$\text{p}K_{\text{A}}$	3.74	3.79	3.89
$\text{p}K_{\text{P}}$	6.04	6.19	6.31
$\text{p}K_{\text{AlAH}}$		3.16	3.38
$\log K_1$	6.17	7.82	7.92
$\text{p}K_{\text{AlA}}$	4.15	4.88	5.46
$\log K_2$	4.18	4.34	4.55
$\text{p}K_{\text{AlA}_2}$		7.15	7.63

^a Table taken from Kiss et al. [25]. Constants are given as logarithms at 25 °C and 0.2 M ionic strength controlled with KCl. Acidity constants are concentration constants. The numbers in parentheses represent one standard deviation in the last digit(s) reported. Upper half of table contains $\log \beta$ values, and lower constants are defined in the text.

^b Precipitation.

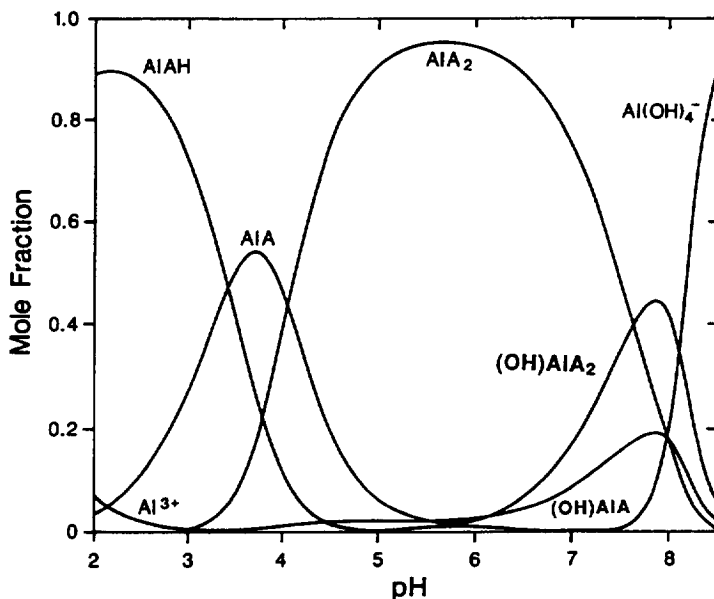


Fig. 1. Plot of mole fraction, Al^{3+} basis, vs. pH for a solution containing 0.1 mM total aluminum and 10 mM total 5'-ATP using the constants in Table 1. The A refers to tetraanionic ATP^{4-} .

The difficulty in determining reliable stability constants is readily apparent from Fig. 1. The coordination chemistry in aqueous solutions containing Al^{3+} and nucleoside phosphates tends to be quite complex and difficult to sort out experimentally. There are several reasons for this: (1) ternary (monomeric) complexes with hydroxide ion exist, the precise nature of which is very pH dependent, (2) true dimeric complexes (such as $[(\text{HO})\text{Al}(\text{ADP})_2]_2$) exist, and their formation is critically dependent on pH, the absolute concentrations of Al^{3+} and nucleoside phosphates and the relative concentrations of Al^{3+} and nucleoside phosphates, and (3) Al^{3+} -bridged complexes (such as $[(\text{HO})_2\text{Al}(\text{ADP})_2]$) exist, and their formation also depends critically on pH and concentration conditions. Potentiometric titrations have provided a wealth of information on the nature of various nucleoside phosphate- Al^{3+} species which can exist in solution under a given set of relatively dilute conditions, as well as values for their complex stability constants [19,25]; however, it is now clear that in more concentrated solutions (where dimers or higher polymers may form) the potentiometric titration approach alone cannot distinguish between all of the possible nucleoside phosphate- Al^{3+} species that are relevant to the speciation problem.

2. Binary complexes of Al^{3+} with nucleoside di- and triphosphates

^1H and ^{31}P NMR spectroscopy provides the best experimental option to sort out nucleoside phosphate- Al^{3+} species present under any given set of conditions. Based

on recent studies by Nelson and Martin [26] and Wang et al. [27], it is clear that at least six species need to be considered at total $[\text{Al}^{3+}]$ to [nucleoside phosphate ligand (A)] ratios of 1 or less (the proportions, of course, will depend on the absolute and relative concentrations of metal and ligand as well as on the pH). These species are A (free ligand; for example, either ADP or ATP), AIA (the 1:1 aluminum ligand complex), HOAIA (with an aluminum ion bound hydroxide, resulting from hydrolysis of the 1:1 AIA complex), $(\text{HOAIA})_2$ (dimer of the hydrolyzed 1:1 complex), $(\text{HOAIA})_2\text{H}$ (protonated dimer of the hydrolyzed 1:1 complex), and AIA_2 (an aluminum ion bridged complex). Experimental analysis of speciation may further be complicated by the fact that three of the above-mentioned species ($(\text{HOAIA})_2$, $(\text{HOAIA})_2\text{H}$, and AIA_2) may exist in open and closed forms, the latter of which is characterized by base “stacking” effects, easily detected by upfield shifts of base protons in proton NMR spectra. How many of these species are actually realized in NMR spectra?

Wang et al. [27] explored the full range of adenosine nucleoside phosphate: Al^{3+} complexation by performing ^1H and ^{31}P NMR spectroscopy on solutions containing ADP and Al^{3+} at various concentrations in varying ratios and at varying pH. Fig. 2, abstracted from Wang et al. [27], presents ^1H NMR and ^{31}P NMR spectra of solutions containing ADP and Al^{3+} at various concentration ratios at pH 6.0. In the ^1H NMR spectra only the aromatic H8 and H2 resonances are shown, while the ^{31}P NMR spectra show the α - and β -phosphate region. Spectrum a in Fig. 2 is the

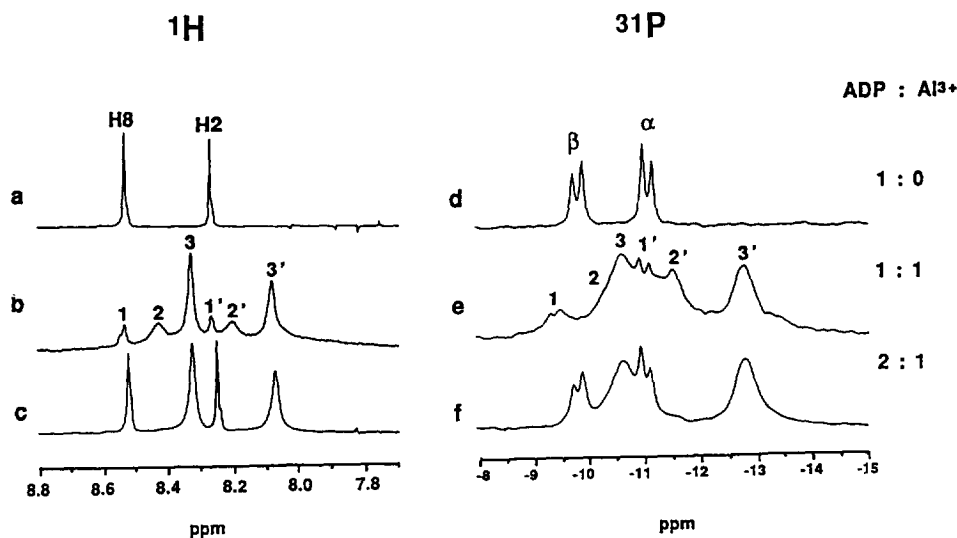


Fig. 2. ^1H (aromatic region) and ^{31}P (α and β phosphate region) NMR spectra of ADP in the absence (spectra a and d) and presence (spectra b, c, e and f) of varying amounts of Al^{3+} at pH 6.0. The concentration of ADP was 10 mM for all spectra except spectra c and f, where the concentration was 20 mM. H8 and H2 in ^1H NMR spectrum a refer to proton positions on the adenine ring. All NMR spectra were acquired at 25 °C on a Varian Unity series 300 MHz NMR spectrometer. Spectra were processed using VNMR (version 3.2a) software implemented on a SUN 4-65 computer.

^1H NMR spectrum of ADP (10 mM) in the absence of Al^{3+} . Spectrum b in Fig. 2 was obtained following the addition of one equivalent of Al^{3+} to the ADP (10 mM) sample, and shows three resolved H8 signals at low field (labeled 1, 2 and 3), two of which (2 and 3) are significantly broadened and upfield shifted relative to the corresponding H8 signal associated with free ADP (spectrum a). Spectrum b also shows three resolved H2 signals at high field (1', 2' and 3'), two of which (2' and 3') are broadened and upfield shifted relative to those of free ADP. (The linkage of resonance signals 1, 2 and 3 to the H8 proton and the resonance signals 1', 2' and 3' to the H2 proton was confirmed [27] by 2D ^1H nuclear Overhauser effect spectroscopy (NOESY) NMR spectra.) Karlik et al. [28], in proton NMR experiments carried out at lower field using ATP as the nucleoside, originally described the splitting of both the H8 and the H2 peaks into multiple peaks around neutral pH (at a nucleoside to Al^{3+} ratio of 1:1). Karlik et al. placed the origin of the upfield-shifted sets of observed proton signals in base stacking interactions, rather than in metal ion coordination to a particular site on the base (for example to an adenine nitrogen atom), since no preferential chemical shift effect on H8 and H2 was evident (i.e. both H8 and H2 peaks are shifted about equally into two new sets of peaks). This would not be expected if Al^{3+} were to bind at one particular site on the base. (The effect on ^1H NMR spectra of metal ion (i.e. Ni^{2+} , Cu^{2+} and Zn^{2+}) binding to N1 and/or N7 nitrogen atoms of purine bases, purine nucleosides and purine nucleoside monophosphates was comprehensively investigated by Martin [29].)

Wang et al. [27] proposed that at a 1:1 molar ratio of ADP (10 mM) to Al^{3+} (spectrum b in Fig. 2), the major species (associated with the 3–3' signals) is $[(\text{HO})\text{Al}(\text{ADP})_2]_2$, a base-stacked, intermolecular, dihydroxy-di- Al^{3+} bridged dimeric structure with each of the two Al^{3+} ions bidentately coordinated to the α - and β -phosphates of a single (but different) ADP molecule and to two hydroxide ions (corresponding to the “closed” form of model species $(\text{HOAlL})_2$ presented at the beginning of this section). Resonance set 2–2' (present at pH 6.0, but not at pH 7.0) was attributed to a $\text{HOAl}_2(\text{ADP})_2$ dimeric, base-stacked species with a single Al^{3+} bridging hydroxide ion (i.e. a monohydroxy-di- Al^{3+} bridged ADP dimer, corresponding to the $(\text{HOAlL})_2\text{H}$ model species).

The 2:1 ADP: Al^{3+} spectrum (spectrum c in Fig. 2) clearly shows only two sets of H8 and H2 proton signals. Wang et al. [27] interpreted the pair of broad upfield-shifted peaks as arising from the bisbidentate $\text{Al}(\text{ADP})_2$ complex, in which two adenine rings are optimally positioned for stacking interactions. (Wang et al. [27] found excellent agreement between the measured integrated area of the broad peaks and the calculated mole fraction of the $\text{Al}(\text{ADP})_2$ complex (fractional integral from the NMR spectrum was 0.65 vs. a mole fraction of $\text{Al}(\text{ADP})_2$ from computer calculation of 0.63).) The computer calculations employed used formation constants presented in Table 1 and procedures as described in Nelson and Martin [26]. (Note that Laussac and Commenges [30], using a low field Brüker WH-90 NMR instrument to collect ^1H , ^{13}C , ^{31}P and ^{27}Al spectra on solutions containing Al^{3+} and ATP at various molar ratios and over a range of pH and temperature, proposed the existence of both the dimer and the bisbidentate species discussed above and sug-

gested that base stacking was the principal stabilizing force. The low sensitivity and resolution of the 90 MHz NMR instrument precluded a detailed multiple equilibrium analysis of speciation using the NMR data; however, this paper remains an insightful and important work illustrating the early application of multinuclear NMR to the difficult problem of Al^{3+} nucleoside phosphate complexation.)

The ^{31}P NMR data on ADP-Al^{3+} systems, presented by Wang et al. [27], supported their conclusions drawn from the ^1H NMR data. The ^{31}P NMR spectrum of ADP (10 mM, pH 6.0), in the absence of Al^{3+} , is shown in spectrum d of Fig. 2. At pH 6.0 the doublet signals from the α and β phosphorus atoms appear at -11.12 ppm (from H_3PO_4) and -9.87 ppm respectively. Spectra e and f in Fig. 2 were obtained following the addition of one and two equivalents respectively of Al^{3+} to the ADP sample. As was observed in the ^1H NMR spectrum for the 1:1 ADP:Al^{3+} sample system (spectra a–c in Fig. 2), the ^{31}P NMR spectrum shows multiple sets of signals. There are three β signals (labeled 1, 2 and 3) and three α signals (labeled 1', 2' and 3'). Clearly, the phosphorus results (spectrum e) correlate extremely well with the proton results (spectrum b). All sets of ^{31}P signals in spectrum e arise almost exclusively from Al^{3+} –ADP complexes, as there is little evidence in spectrum e for the presence of free ADP (i.e. there is no appreciable signal at the resonance position of the β phosphorus of free ADP). Computer calculations predicted that the major species (associated with ^{31}P resonance set 3–3') has the stoichiometry $\text{HOAl}(\text{ADP})$, consistent with base-stacked, dimeric $[(\text{HO})\text{Al}(\text{ADP})]_2$. ^{31}P resonance set 2–2' was attributed to the base-stacked monohydroxy-di- Al^{3+} bridged ADP dimer, $\text{HOAl}_2(\text{ADP})_2$. (Note that resonance set 2–2' virtually disappears as the pH is raised from 6.0 to 7.0, a finding consistent with the above interpretation.)

The 2:1 ADP:Al^{3+} ^{31}P NMR spectrum (spectrum f in Fig. 2) clearly shows only two sets of α and β phosphorus signals. ^{31}P signals 2 and 2' which were present in spectrum e are essentially absent in spectrum f. In addition, the β doublet present in spectrum e has moved upfield (to a position essentially identical to that expected for the β signals in free ADP) in spectrum f. The data presented in spectrum f of Fig. 2 are consistent with the bisbidentate $\text{Al}(\text{ADP})_2$ complex. The ^{31}P NMR results (spectrum f in Fig. 2) on the 2:1 ADP:Al^{3+} sample are remarkably similar to the ^1H NMR results (spectrum c in Fig. 2).

The ^{31}P NMR results of Wang et al. [27] on ADP are consistent with the earlier ^{31}P NMR results of Laussac and Commenges [30] on ATP, in that Al^{3+} binding to the nucleoside caused upfield shifts for all phosphorus resonances. This is in contrast to the downfield chemical shifts produced by a variety of diamagnetic, divalent metals, including Mg^{2+} , Ca^{2+} , Zn^{2+} and Cd^{2+} , when complexed to the phosphate oxygens. (The ^1H NMR upfield shifts observed for the Al^{3+} –nucleoside complexes almost certainly arise from electronic factors associated with base stacking interactions. While upfield ^{31}P NMR shifts, of the type observed in this study, are usually attributed to Al^{3+} ion-induced alterations in the oxygen–phosphorus–oxygen torsional angles, the rather remarkable coincidence that the proton and phosphorus spectra appear so similar may suggest that an electronic shielding component is also important in determining the observed ^{31}P NMR shifts.) Further consistent with the work of Wang et al. [27], ^{31}P NMR signals arising from both the dimeric $[\text{Al}(\text{ATP})]_2$

species and the bisbidentate $\text{Al}(\text{ATP})_2$ species were identified in the ^{31}P NMR spectra of Laussac and Commenges [30]. From the magnitudes of the upfield chemical shifts observed, it was inferred that, for both species, chelation involved primarily the P_β and P_γ groups. (The ^{27}Al NMR line widths $\nu_{1/2}$ for the dimeric $[\text{Al}(\text{ATP})]_2$ species and the bisbidentate $\text{Al}(\text{ATP})_2$ species were measured, by Laussac and Commenges [30], to be 600 Hz and 2.4 kHz respectively, interpreted by these researchers to indicate a substantial distortion (i.e. lowering of the octahedral symmetry) of the β – γ bidentate chelate rings in the latter species.)

A major distinction between the work of Karlik et al. [28], using ATP as the nucleoside, and Wang et al. [27], using both ADP and ATP (see Ref. [31]) as nucleosides, has to do with the interpretation of the most upfield-shifted H8 and H2 peaks in 1:1 solutions of nucleoside phosphate and Al^{3+} at relatively high concentrations (i.e. about 10 mM) in the pH 6–7 range. Karlik et al. [28] suggested that the upfield-shifted resonance signals derived from a species (referred to as “complex III” in their paper) best described as a single, polymeric structure containing three different types of base stacking (the precise nature of which was left, however, rather vague). The dimeric interpretation of Wang et al. [27] (i.e. dimeric $[(\text{HOAl}(\text{ADP}))_2]$, associated with the most upfield-shifted resonance set 3–3', and $[(\text{HO})\text{Al}(\text{ADP})]_2\text{H}$, associated with the upfield-shifted resonance set 2–2') represents a much simpler explanation of both the ^1H and ^{31}P NMR data and, furthermore, is quite amenable to experimental verification.

If the dimeric model of Wang et al. [27] (and others) is valid, then one might expect that dilution of the relatively concentrated 10 mM ADP, 10 mM Al^{3+} sample would result in disruption of the dimeric structure. Such a structural collapse should be easily detected by proton NMR. Figs. 3(a) and 3(b) present 500 MHz ^1H NMR spectra associated with progressive dilution of 1:1 ADP: Al^{3+} mixtures, from 10 mM ADP down to 0.5 mM ADP at pH values of 6.0 and 7.0 respectively. (The analogous spectra for ATP are shown in Figs. 4(a) and 4(b).) As predicted, the ^1H NMR spectra change progressively and dramatically with dilution. Dilution-induced collapse of the stacking interactions in bisbidentate $\text{Al}(\text{ADP})_2$ complexes at pH values of 6.0 and 7.0 is indicated in the series of spectra shown in Figs. 3(c) and 3(d) respectively. (The analogous spectra for ATP are shown in Figs. 4(c) and 4(d).) Computer simulations performed by Martin and coworkers [31] on the ADP data presented in Fig. 3 and the ATP data presented in Fig. 4 indicate that reasonably good fits can be obtained only for dimer–monomer equilibria. The calculations clearly indicate that it is unlikely that multimeric complexes higher than dimeric exist under the experimental conditions employed by either Wang et al. [27] or Karlik et al. [28].

Two additional general observations can be made from the NMR results presented in Figs. 3 and 4. First, the ADP spectra corresponding to highest dilution (i.e. the topmost spectra in Figs. 3(a)–3(d)) are all quite similar (the only exception being the upfield-shifted H8 peak at about 8.4 ppm in Fig. 3(a), attributed to a hydroxide-deficient monomeric $\text{ADP}-\text{Al}^{3+}$ species). The ADP dilution experiments indicate that at high concentrations (about 10–20 mM) a substantial percentage of ADP molecules are involved in stacking interactions at both ratios of ADP to Al^{3+} and at both pH values, while at low ADP concentrations (about 0.5–1 mM) a rather

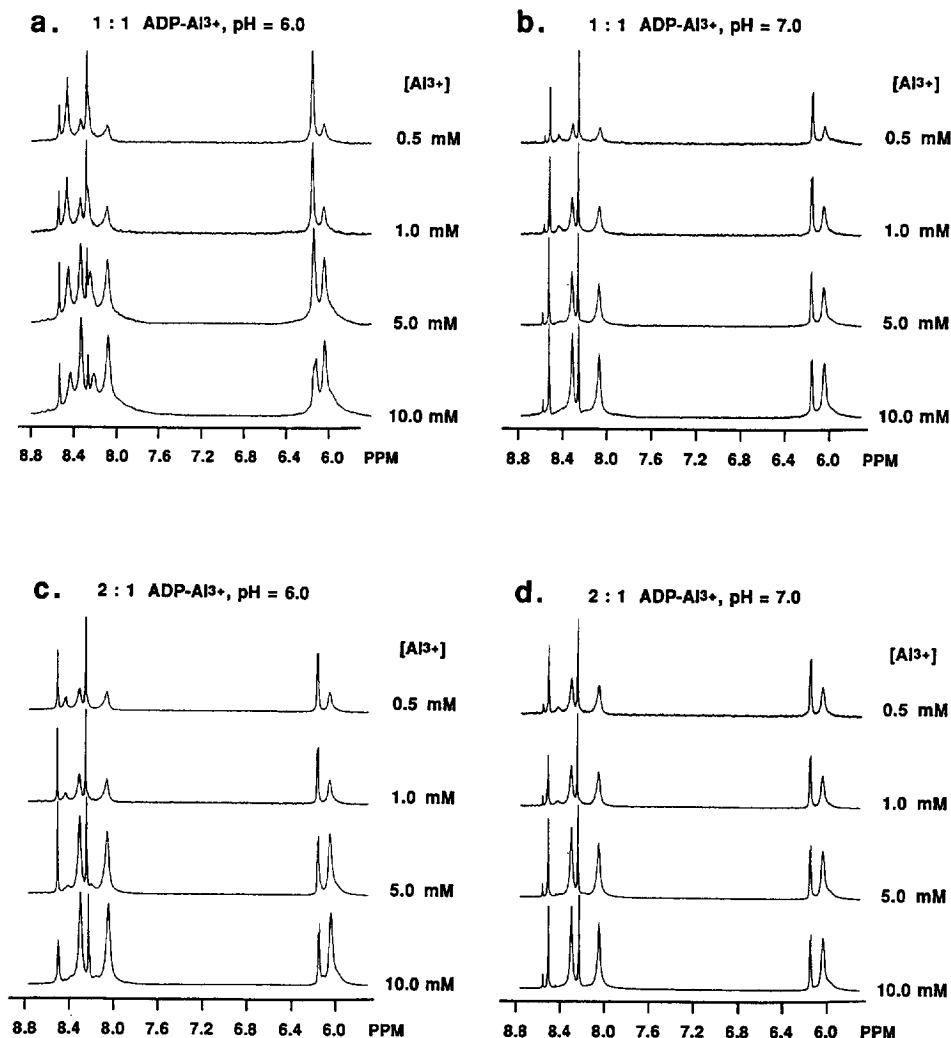


Fig. 3. ^1H NMR spectra of 1:1 and 2:1 complexes of ATP and Al^{3+} at pH values of 6.0 and 7.0 as a function of concentration. Only the downfield portions of the spectra are shown. The H_1' sugar proton resonance appears in the 5.8–6.2 ppm range, and the aromatic H8 and H2 adenine ring resonances appear in the 7.8–8.8 ppm range. All NMR spectra were acquired at 25 °C on a Varian Unity series 500 MHz NMR spectrometer. Spectra were processed using VNMR (version 3.2a) software implemented on a SUN 4-65 computer.

limited percentage of ADP molecules are involved in stacking interactions at both ratios of ADP to Al^{3+} and at both pH values. Second, the ATP dilution results are significantly different from the ADP dilution results. The ATP dilution experiments clearly indicate that there is less tendency for ATP to be involved in stacking interactions at a 2:1 ratio of ATP to Al^{3+} than at a 1:1 ratio of ATP to Al^{3+} (at

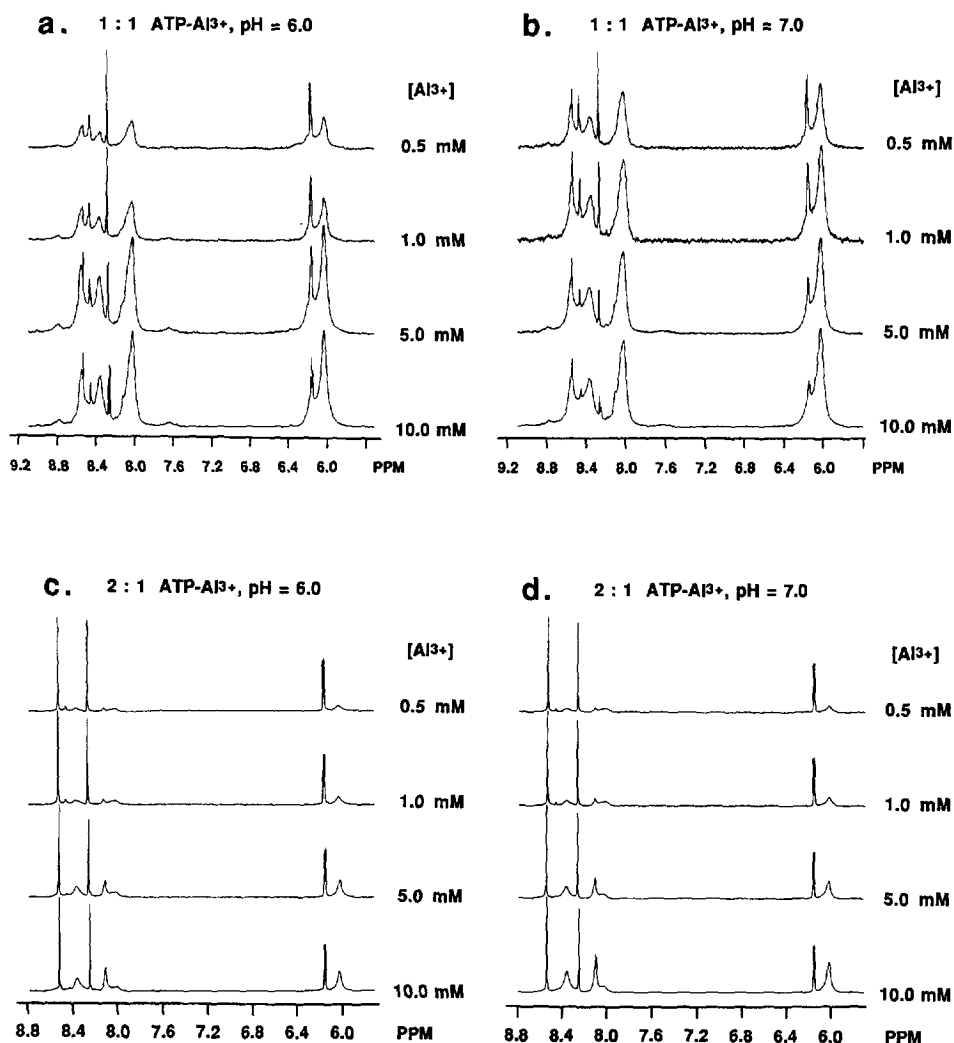


Fig. 4. ^1H NMR spectra of 1:1 and 2:1 complexes of ADP and Al^{3+} at pH values of 6.0 and 7.0 as a function of concentration. Only the downfield portions of the spectra are shown. The H_1 sugar proton resonance appears in the 5.8–6.2 ppm range, and the aromatic H8 and H2 adenine ring resonances appear in the 7.8–8.8 ppm range. All NMR spectra were acquired at 25°C on a Varian Unity series 500 MHz NMR spectrometer. Spectra were processed using VNMR (version 3.2a) software implemented on a SUN 4-65 computer.

both high and low concentrations of ATP and at both pH 6.0 and pH 7.0). Comparison of Figs. 3(c) and 3(d) with Figs. 4(c) and 4(d) further indicates that at a 2:1 ratio of nucleoside to Al^{3+} there is less tendency for ATP to stack at all concentrations employed and at both pH values examined than for ADP to stack.

3. Ternary complexes with fluoride ion

Speciation in systems containing nucleoside diphosphates, Al^{3+} and F^- is of biochemical interest since it has been proposed that ternary complexes of these three species can mimic nucleoside triphosphates in the premature activation of G protein systems, which can potentially lead to disfunction in biological systems [15,16,32,33]. Prediction of which complexes are most relevant in G protein systems near neutral pH is complicated by the amphoteric nature of aluminum giving rise to the expected presence of mixed hydroxide species. As a logical first step in the analysis of relevant species, Martin [34] examined ternary complexes of Al^{3+} , F^- and OH^- in neutral aqueous solutions (in the absence of nucleosides) and found evidence for the existence of at least five different mixed OH^- and F^- complexes of Al^{3+} . It has been claimed by numerous researchers that the tetrahedral species, AlF_4^- , mimics tetrahedral phosphate and thus serves as the primary activating species in G protein systems. However, Martin [34] indicated that AlF_4^- exists as a hexacoordinate species in aqueous solution with two bound water molecules. Kiss et al. [25] extended the initial analysis of Martin [34] to include a study of the complexes formed between various nucleosides (i.e. AMP, ADP, and ATP) and the Al^{3+} ion, and Nelson and Martin [26] went on to investigate ternary complexes formed from nucleoside diphosphates (NDP, including both GDP and ADP), Al^{3+} and F^- . In the latter study, ^{19}F and ^1H NMR identified the ternary complexes $(\text{NDP})\text{AlF}_x$ ($x = 1-3$), but no $(\text{NDP})\text{AlF}_4$ was found. The ternary complex $(\text{NDP})\text{AlF}$ appeared with an abundance that was equal to that expected statistically on the basis of binary complex stabilities. However, under the conditions of maximum effect in G protein experiments in neutral solutions, most Al^{3+} occurred as $(\text{HO})\text{AlF}_3^-$. In the work of Nelson and Martin [26] unknown stability constants of ternary species were determined from best fits of computer-generated curves for each species to the experimentally determined mole fractions (as measured from the ^{19}F NMR spectra). NMR experiments were performed on both ADP and GDP and over 30 species were included in the comprehensive theoretical analysis. The computer procedures developed allowed these researchers to define the species present in aqueous solution under a wide variety of conditions of pH and Al^{3+} , NDP, and F^- concentrations. The effect of increasing fluoride ion concentration on nucleoside speciation in solutions containing ADP– Al^{3+} molar ratios of 1:1 and 2:1 (at pH values of 6.0 and 7.0) is shown in Fig. 5. The plots presented in Fig. 5 were generated using equilibrium constants presented in Table 1 as well as ternary formation constants presented in Nelson and Martin [26]. The four parts of Fig. 5 correspond exactly to the four parts of Fig. 3 (which presents ^1H NMR spectra at ADP– Al^{3+} molar ratios of 1:1 and 2:1, at pH values of 6.0 and 7.0). Comparison of the plots in Fig. 5 indicates that the non-fluoride-ion containing species most dramatically affected by increasing fluoride ion concentration are the 1:1 $(\text{OH})\text{Al}(\text{ADP})$ and the bisbidentate $\text{Al}(\text{ADP})_2$ species. The most prevalent fluoride ion containing species, in all cases, is predicted to be $\text{F}_2\text{Al}(\text{ADP})$. Ternary complexes of nucleoside phosphates, Al^{3+} and fluoride ion are discussed later in this paper (in the context of G proteins) and elsewhere in this volume (see Martin [35]).

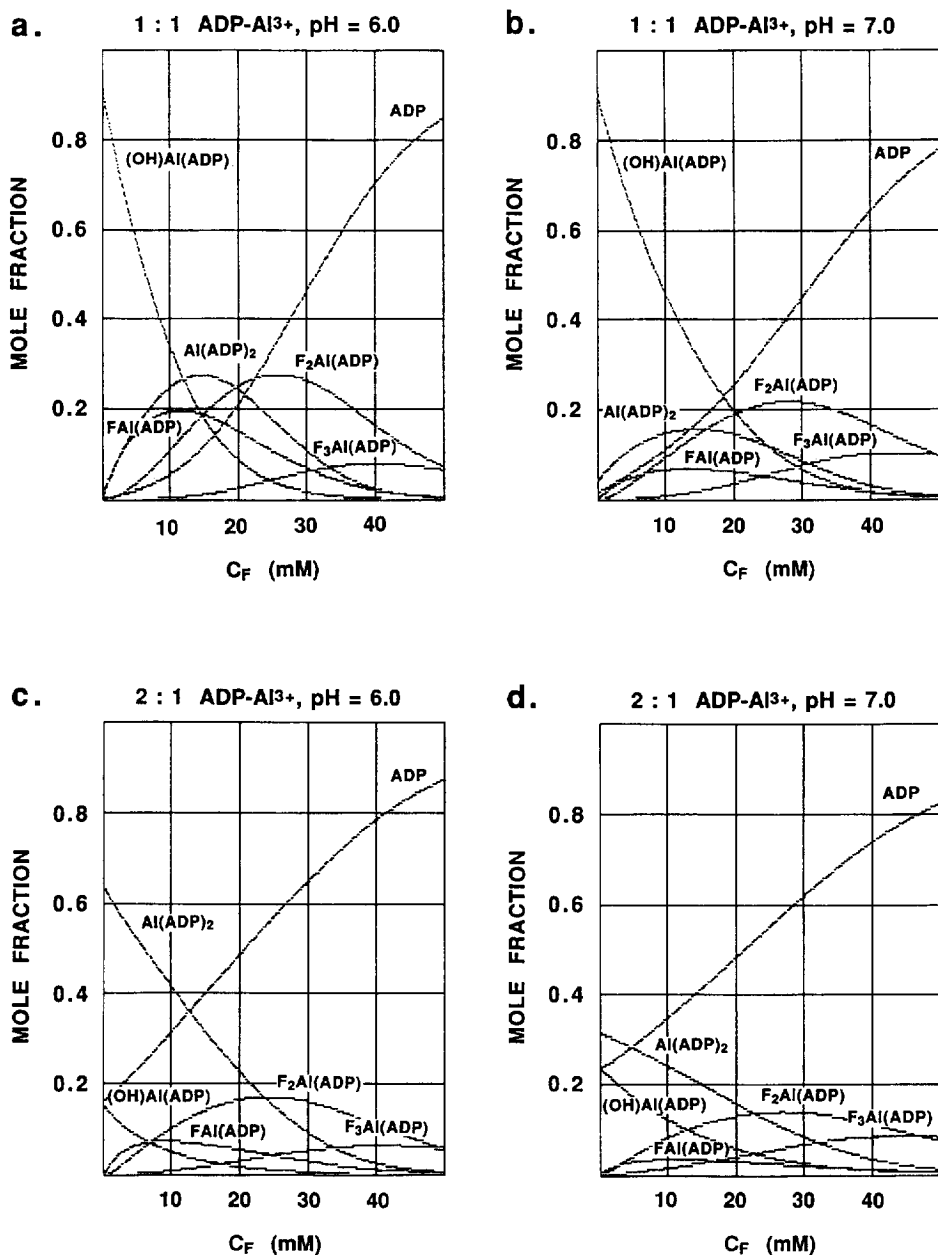


Fig. 5. Species distribution plots on a mole fraction phosphate basis for 1:1 and 2:1 complexes of ADP- Al^{3+} at pH values of 6.0 and 7.0 as a function of total fluoride ion concentration. The four plots presented correspond to the same experimental conditions presented in Fig. 3.

4. Al^{3+} complexes with non-nucleoside-binding proteins

Aluminum ion binds to a wide variety of proteins. In the case of non-enzymatic metalloproteins, the Al^{3+} most often binds to the same site as the naturally occurring metal ion. The extracellular iron transport protein, transferrin, is a particularly relevant example. Transferrin is the principal protein target for Al^{3+} in blood plasma, and Savory and coworkers, using difference spectrometry, have determined the associated stability constant [36]. (Earlier in this review it was mentioned that citrate is the principal (small molecule) carrier of Al^{3+} in plasma. Martin [37] has estimated a 6 h half-life for the transfer of Al^{3+} from plasma transferrin to citrate (at a plasma concentration of 0.1 mM, pH 7.4).) While Al^{3+} clearly has high affinity for phosphate groups, it is interesting that the major protein binder for Al^{3+} in plasma (i.e. transferrin) does not use phosphate coordination, but rather three anionic oxygen atoms from two tyrosyl phenolates and one aspartyl side chain, and a single neutral nitrogen atom from a histidine imidazole ring [38]. As pointed out by Martin [37], transferrin affords a convenient mechanism for the transfer of neurotoxic Al^{3+} into the central nervous system, since the transferrin receptor recognizes surface features of transferrin and not the naturally bound Fe^{3+} . The discussion of Al^{3+} binding to transferrin is relevant to the central theme being presented in this review, since transferrin (and citrate for that matter) may play a critical role in transporting Al^{3+} to biological compartments where subsequent transfer to nucleoside phosphates (either free or protein bound) may occur.

An important non-transport, non-enzymatic metalloprotein purported to bind Al^{3+} is the ubiquitous, intracellular Ca^{2+} ion receptor, calmodulin. (Calmodulin is particularly abundant in brain and while not an enzyme itself it has the ability, following complexation to Ca^{2+} , to activate a number of enzymes critical to the neurotransmission process.) A series of papers by Haug and coworkers [39,40] indicated that Al^{3+} binds tightly (i.e. $K_d \approx 0.4 \mu\text{M}$) to calmodulin, inducing a significant conformational change (an approximate 30% reduction in α -helical content) in the protein. Haug and coworkers noted that “a molar ratio of 4:1 for [aluminum]/[calmodulin] is sufficient to block completely the activity of the calcium calmodulin-dependent phosphodiesterase” [40]. These results were challenged by Richardt et al. [41], in which it was concluded that “aluminum ions ... do not interact with calmodulin,” and that aluminum “... neither substituted for Ca^{2+} in activating calmodulin-dependent phosphodiesterase nor inhibited binding of this ion to calmodulin, and failed to mimic the effects of a typical calmodulin antagonist like chlorpromazine.” Weis and Haug [42] later showed that the Al^{3+} binds at sites other than the Ca^{2+} ion binding sites. A later work by You and Nelson [43], using electron paramagnetic resonance spectroscopy to study Al^{3+} binding to tyrosine-99 spin-labeled bovine brain calmodulin, found little evidence for strong binding of Al^{3+} to the protein at the Ca^{2+} binding sites (not surprising since the volume of Al^{3+} (in its favored 6-fold coordination) is approximately nine times less than Ca^{2+} (in its favored 8-fold coordination) [6]). (Note that tyrosine-99 provides a carbonyl ligand to a metal ion coordinated at one of the four Ca^{2+} binding sites in calmodulin, and the binding of many metal ions, including Ca^{2+} , Cd^{2+} and Tb^{3+} , all affect the

EPR spectrum quite dramatically.) As with transferrin, discussion of calmodulin is relevant to this Al^{3+} -nucleoside review since the protein is particularly abundant in tissues (i.e. brain) known to accumulate Al^{3+} , and thus, if Al^{3+} does interact with calmodulin, the protein may serve as a transient target for Al^{3+} on its way to a high affinity nucleoside binding site (say on a G protein). Haug and Vitorello [44] review Al^{3+} interaction with calmodulin and the physiological significance of this potential association elsewhere in this volume.

5. Al^{3+} complexes with nucleoside-binding proteins

In the case of nucleoside-requiring enzymes, the typical response of Al^{3+} complexation is inhibition of enzyme activity (although activation can sometimes result, depending on conditions), with Al^{3+} binding at an essential metal ion binding site in the protein. (It is possible, however, that Al^{3+} may inhibit (or activate) by binding at a site other than a metal ion binding site, such as at one or more non-phosphorylated or phosphorylated amino acid side chains, or to a substrate-cofactor. Also, in some instances the Al^{3+} response is linked to the presence of an additional non-protein ligand (e.g. fluoride ion) (see next section).) As pointed out earlier in this review, Al^{3+} can serve as an effective Mg^{2+} surrogate. If an enzyme requires Mg^{2+} for activity, Al^{3+} is likely to interfere with (usually inhibit) normal function. Martin [6] and Macdonald et al. [5] point out that Al^{3+} inhibition of enzyme activity is likely to have both kinetic and thermodynamic bases. For example, in the case of ATP^{4-} -utilizing kinases, Al^{3+} -ligand (i.e. ATP^{4-}) exchange rates tend to be exceedingly slow (about 10^5 -fold slower for Al^{3+} than for Mg^{2+}), and Al^{3+} binding affinity to ATP^{4-} tends to be exceptionally high (about 10^7 times higher for Al^{3+} than for Mg^{2+}). Thus, the cofactor ATP^{4-} binds to the enzyme (most probably through an Al^{3+} bridge), but the turnover is exceptionally low, and the kinase is potentially inhibited. The first Mg^{2+} -dependent enzyme shown to be inhibited by Al^{3+} was, in fact, a kinase, specifically hexokinase [20,45]. Other Mg^{2+} -dependent enzymes involved in phosphate transfer, such as 3',5'-cyclic nucleotide phosphodiesterase and the acid and alkaline phosphatases, have also been shown to be inhibited by Al^{3+} [46,47]. Furumo and Viola [48] have examined Al^{3+} inhibition of a large number of phosphoryl-transferring enzymes, and have concluded that those enzymes (such as yeast hexokinase and bacterial glycerokinase) which preferentially bind the Λ β,γ -bidentate isomer of the Al^{3+} -ATP complex exhibit the strongest inhibition. (The Λ isomer consists of a right-handed helix about an axis drawn through the metal ion perpendicular to the chelate ring.) Esterases (acetylcholinesterase and serum cholinesterase), oxidases (ferroxidase), and non-phosphate transferases (catechol-*O*-methyl transferase) have also been reported to be inhibited by Al^{3+} . Adenylate cyclase is an example of an enzyme which can be both inhibited and activated by Al^{3+} , depending on precise conditions. Further details on Al^{3+} perturbation of the normal function of these and other Mg^{2+} -dependent enzymes can be found in the work by Macdonald and Martin [46] and in the compilation by Ganrot [47].

An example of a non-enzymatic, nucleoside-binding protein positively affected by

Al^{3+} is tubulin. Al^{3+} has been shown by Macdonald et al. [5] to promote tubulin assembly into microtubules. In this “activation” process Al^{3+} once again substitutes for Mg^{2+} , in this case at the “exchangeable” nucleotide site on tubulin subunits. While Al^{3+} “activates” the tubulin polymerization process, it strongly inhibits the GTPase activity of the microtubule, for the same kinetic and thermodynamic reasons (as a consequence of the much higher charge density on Al^{3+} relative to Mg^{2+}) that were discussed above in the context of Al^{3+} inhibition of kinases [6,46].

6. Aluminofluoride complexes with nucleoside-binding proteins

Perhaps the most actively studied proteins that are affected by Al^{3+} are the guanosine nucleotide-binding proteins (G proteins) [49]. One member of this class of “signal transduction” proteins is transducin (or G_v , rod photoreceptor GTP-binding protein), which has been examined in great detail by Macdonald and coworkers. Miller et al. [17] have shown that Al^{3+} ion alone inhibits the GTPase activity of transducin principally by inhibiting receptor-catalyzed nucleotide exchange (i.e. fewer molecules of GTP bind to transducin). In marked contrast, Al^{3+} in the presence of fluoride ion inhibits the GTPase activity by binding (as an fluoroaluminate species, AlF_x where x is probably 3 [26,35]) to the Mg^{2+} –GDP–transducin complex. (The binding of the AlF_x species to the G protein presumably occurs at the site normally occupied by the γ -phosphate of activating GTP, giving rise to an Mg^{2+} –GDP– AlF_x complex which cannot be catalytically hydrolyzed with measurable efficiency.) The effect of Al^{3+} on signal transduction in the transducin system in the absence and presence of fluoride ion is also totally different. In the absence of fluoride ion, Al^{3+} causes overall signal transduction to decrease (because less GTP binds) while, in the presence of fluoride ion, Al^{3+} markedly enhances signal transduction (i.e. the Mg^{2+} –GDP– AlF_x complex effectively mimics the activating Mg^{2+} –GTP species).

A ^{31}P and ^{19}F NMR study by Higashijima et al. [50], also utilizing transducin, has provided support for the above mechanism of fluoroaluminate inhibition of G protein GTPase activity (and activation of G-protein-mediated signal transduction). In the presence of Al^{3+} and fluoride ion, the β phosphorus signal (of GDP) in the ^{31}P NMR spectrum of G-protein-bound GDP shifted to a position similar to the β phosphorus signal of GTP. In addition, the ^{19}F NMR spectral data on the complex were most consistent with the $\text{GDP} \cdot \text{AlF}_3$ being the most relevant protein bound nucleoside species, consistent with more recent fluoroaluminate speciation studies [26,51,52]. (A recent ^{31}P NMR and fluorescence study by Antonny et al. [53] has demonstrated an Al^{3+} -independent mechanism of G protein activation (i.e. enhanced signal transduction) by fluoride ion and Mg^{2+} . The postulated G protein bound nucleoside complex is Mg^{2+} –GDP– MgF_3 , with the MgF_3 again occupying a position normally held by the γ -phosphate of activating GTP.)

7. Conclusion

Application of refined potentiometric methods and the full range of multinuclear NMR spectroscopic techniques to the difficult problem of complexation in binary systems containing Al^{3+} and nucleoside phosphates, as well as to the much more complex ternary and quaternary systems with fluoride ion and nucleoside-binding proteins, has yielded a wealth of speciation information. With the realization that Al^{3+} is an extremely effective surrogate for Mg^{2+} and with a better understanding of the manner in which both Mg^{2+} and Al^{3+} complex with the phosphate chain of nucleoside di- and triphosphates, the thermodynamic and kinetic bases for Al^{3+} -induced inhibition and activation of a number of protein-based processes are now reasonably well understood. NMR spectral data of sufficient quality are now being obtained to allow determination of equilibrium constants for a number of ternary complexes (for example, ternary complexes involving nucleoside phosphate, Al^{3+} and fluoride ion), and to discriminate between various models for complexation (such as dimer vs. trimer or higher polymer). The major objective in the immediate future will be to obtain atomic level structural information on systems containing nucleoside phosphate, Al^{3+} and nucleoside-binding proteins (perhaps with fluoride ion). Multidimensional, multinuclear NMR spectroscopy should prove extremely useful in this endeavor. A firm foundation has been laid, but much work remains to be accomplished.

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