Multinuclear NMR Study of Fluoroaluminate Complexes in Aqueous Solution

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It has been assumed that the sensitivity of several phosphate-binding enzymes to fluoroaluminate complexes was due to a similarity of the AlF_4^- complex with the tetrahedral phosphate anion. Depending on the stoichiometry, the ligand nature, and the medium conditions, aluminum may form either octahedral or tetrahedral complexes with molecular ligands. An exhaustive NMR (^{27}Al and ^{19}F) study of aqueous solutions of fluoride and aluminum ions from pH 2 to pH 11 and for [F]/[Al] ratios up to 14 permits the characterization of the fluoroaluminate complexes observed. This study provides evidence of complexes at pH $\leq$ 6 containing respectively one and two fluorine atoms per aluminum, the other aluminum ligands being H_2O. Contrary to previous results, assignments of the resonances to complexes corresponding to three or more fluorines per aluminum was difficult over the range of temperatures studied. This is due to rapid exchange processes between H_2O and F^- ligands. At pH higher than 2, it has been possible to assume the existence of complexes binding one or two OH^- per aluminum with three or more fluorines undergoing a fast exchange. A variable-temperature ^{19}F NMR study pinpoints the pH-dependent exchange conditions between OH^- and H_2O ligands for the complexes possessing three or more fluorine atoms per aluminum. The present study also permits the conclusion that all the fluoroaluminate complexes observed in aqueous solution are hexacoordinated with an octahedral geometry. The equilibrium constants K_{AlF_1} to K_{AlF_3} have been experimentally determined.

Introduction

Fluorometallic complexes like fluoroberyllates and fluoroaluminates are known to interfere with the activity of a large variety of nucleotide-binding proteins.[1] Their effects on both G-proteins and P-type ATPases were first displayed in the presence of a nucleoside diphosphate. The most generally accepted explanation of their role was that the fluorides and metal atoms formed a tetrahedral complex resembling a phosphate moiety. This complex with the nucleoside diphosphate was thought to mimic both the shape and the size of a nucleoside triphosphate,[2−4] although this complexation did not occur in solution when beryllium is the metal cation.[5] This assumption has been strengthened by the observation of the fluoroaluminate's ability to inhibit P-type ATPases when interacting with the phosphorylation site of these enzymes, in the absence of any nucleoside diphosphate.[6,7]

When the metal in solution is beryllium, the geometry of the cation coordination sphere is tetrahedral, regardless of the number and the type of the liganding groups (F^-, OH^-, or H_2O). On the other hand, aluminum ions in aqueous solutions are supposed to form either hexa- or tetracoordinated complexes, and the question of the relevant conformation remains controversial. The first NMR experimental studies[8,9] indicated four hexacoordinated products, from AlF_1 to AlF_4. But the experimental conditions were far from the enzyme-inhibiting conditions: high concentrations (1 M) and acid-uncontrolled pH. Recently published results on the structures of G-proteins crystallized with nucleotides and fluoroaluminates suggest that the fluoroaluminate complex present in the active site exhibits an octahedral conformation with four fluoride ions in a square planar configuration.[10,11] However, although theoretical studies[12] exclude any tetracoordination for AlF_x in aqueous solutions, it has been pointed out that ternary species, such as $AlOH_yF_x$, might be tetrahedral.

In the present study, ^{27}Al and ^{19}F NMR investigation has been used to study in detail the assignment of the NMR resonances and the geometry of the fluoroaluminate complexes in aqueous solution. Our results confirm that the octahedral geometry for all detected fluoroaluminate complexes is prevalent under all studied conditions, including those giving rise to an inhibition of the P-type ATPases.

Experimental Section

Materials. The aluminum cation source is aluminum nitrate (Merck), and the fluoride anion sources sodium fluoride (Riedel DeHaen). Aqueous solutions contained 30% D_2O; the pH of all samples was adjusted to within ±0.1 units with a pH meter (corrected for D_2O). The solutions were prepared for a final concentration of 5 mM aluminum and different fluoride concentrations. Measurements were made at least 1 h after sodium fluoride was mixed with aluminum nitrate, in order to get complete thermodynamic equilibrium.

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NMR Measurements and Data Treatment. NMR was performed at 350 K (in order to get sharper resonances)[13] on a Bruker AM400 spectrometer for ^{27}Al and at 276 K on either a Varian U400 or U+500 spectrometer for ^{19}F (unless specified). ^{27}Al spectra were run at 104.26 MHz on a multinuclear probe (10 mm). Typical acquisition parameters were as follows: pulse width corresponding to 60°, relaxation delay = 0.3 s, acquisition size = 8K, and sweep width = 200 ppm. Prior to Fourier transform, the first points were suppressed by left-shifting data for nulling an intense and wide ($\approx$4000 Hz) signal, centered about 60 ppm, generated by the probe,[14] and then apodization was performed. The resulting spectra were baseline corrected. Chemical shifts are given relative to a 0.1 M aluminum nitrate solution (pH = 1). Tentative integration of very broad peaks was performed from a reference capillary tube of an aluminum nitrate solution (pH = 12) according to a protocol described in the literature.[15] ^{19}F NMR spectra were run at 376.3 or 470.26 MHz on a 5 mm indirect detection probe. Typical acquisition parameters were as follows: pulse width corresponding to 50°, relaxation delay = 0.3 s, acquisition size = 6K, spectral width = 50 ppm. Before Fourier transform, zero filling and linear prediction followed by apodization were performed. Chemical shifts were measured relative to trifluoroacetic acid and given relative to the $CFCl_3$ standard reference (−76.6 ppm relative to $CFCl_3$).

Results

Several NMR studies have been made on fluoroaluminate complexes in different media[8,9,12,16−18] and specifically in aqueous solution, but the results are often difficult to analyze, since they do not separate the effects of pH and reactant concentration. Therefore, we carried out an extensive study of fluoroaluminates in aqueous solutions at different pH values and [F]/[Al] ratios monitored by ^{27}Al and ^{19}F NMR.

^{27}Al NMR Spectra. For the spectra measured at pH $\leq$ 4 and [F]/[Al] ratios $\leq$ 5, all the aluminum introduced in the sample could be observed as two signals at room temperature. The first one, at 0 ppm with a line width ranging from 4 to 70 Hz, observed for pH $\leq$ 3 when [F]/[Al] $\leq$ 1, corresponds to the aqueous aluminum complexes.[13] The second resonance, located from 0.5 ppm ([F]/[Al] = 1) to 3 ppm ([F]/[Al] = 6), shows a line width which depends upon the [F]/[Al] ratio. The line width increases from 90 to 300 Hz as the [F]/[Al] ratio increases from 1 to 3, and then decreases to 200 Hz as the ratio increases to 5. There were no observable coupling patterns. At 350 K, these two lines are difficult to separate. The typical resonances of fluoroaluminate complexes are shown in Figure 1.

For [F]/[Al] ratios higher than 5 and values of pH higher than 3, the intensity of the detected signal decreased and is close to zero for [F]/[Al] $\approx$ 11. For values of pH higher than 8, no ^{27}Al resonance was observed, except for that from $Al(OH)_4^-$ at 80 ppm.

^{19}F NMR Spectra. Typical ^{19}F spectrum is shown in Figure 2. Five different resonances, denoted S1 to S5, have been detected from −152.3 to −154.9 ppm. Their characteristics are given in Table 1. The dependence of the signal intensity on fluorine concentration at differents pH's for a constant aluminum concentration (5 mM), is shown in Figure 3. At pH > 8, only the free fluoride signal is observed ($\delta = -120.2$ ppm). For [F]/[Al] ratios higher than 8, the signal intensity of the fluoroaluminate complexes fades away. This behavior occurs at all pH's studied, except at pH 2, where the S2 and S3 signals remain present for [F]/[Al] up to 14. The spectrum measured at pH 2 also includes the signal corresponding to HF ($\delta = -162.6$ ppm).

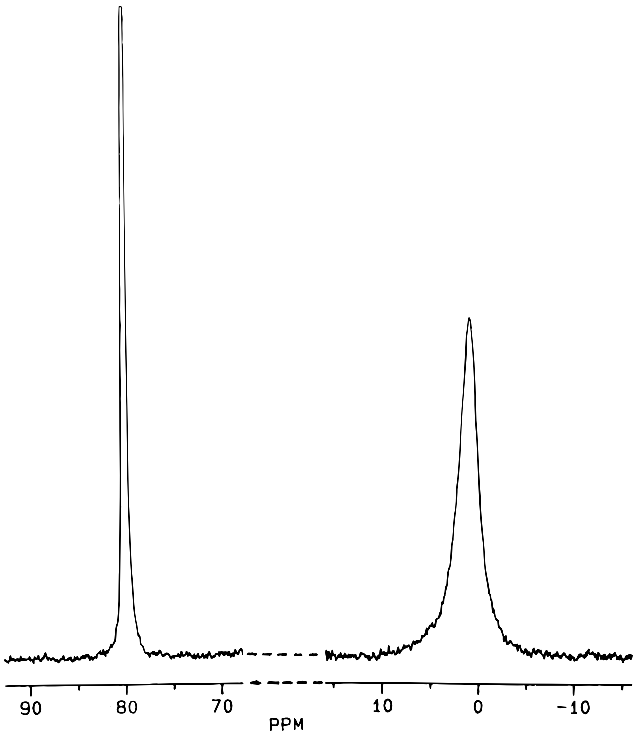

Figure 1. ^{27}Al NMR (104.26 MHz) spectrum of the fluoroaluminate complexes obtained upon mixing 5 mM $Al(NO_3)_3$ and 15 mM NaF at pH 4. The peak at 80 ppm corresponds to $Al(OH)_4^-$ used as external reference.

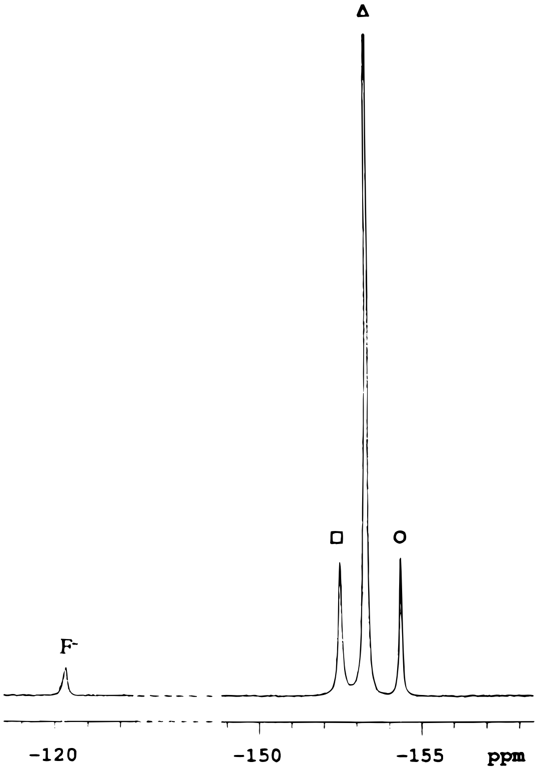

Figure 2. ^{19}F NMR (376.3 MHz) spectrum of the fluoroaluminate complexes obtained upon mixing 5 mM $Al(NO_3)_3$ and 15 mM NaF at pH 4: ○, signal S2; △, signal S3; □, signal S4.

Resonances S1 and S2. Both signals S1 and S2 behave in a comparable manner. They were detected at pH's of 1 and above, and their intensities were highest for stoichiometric ratios

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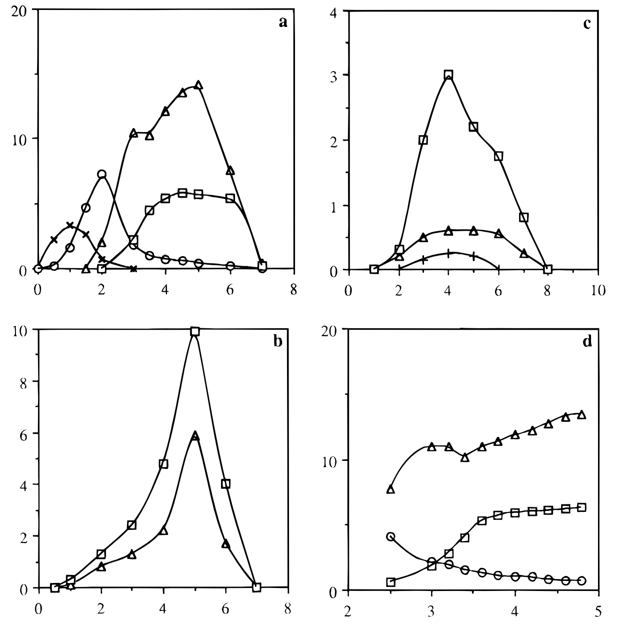

Figure 3. Intensity of the various ^{19}F signals (expressed in millimolar fluorine concentration) *vs* total fluorine/aluminum ratio for 5 mM Al(NO$_3$)$_3$, at various pH values: a, pH 4; b, pH 7; c, pH 8; d, enlargement at pH 5 enlightening the existence of the shoulder of the [F]/[Al] dependence of S3 intensity. ×, signal S1; ○, signal S2; △, signal S3; □, signal S4; +, signal S5. The assignments of the signals are proposed in the text.

Table 1. Characteristics at 276 K of the ^{19}F NMR Signals of the Fluoroaluminate Complexes

	S1	S2	S3	S4	S5
chemical shift (ppm)	−154.9	−154.4	−153.3	−152.6	−152.3
line width (Hz)	19	37	20	30	≈50
assignment (see text)	AlF$_1$	AlF$_2$	AlF$_x$	AlOHF$_x$	AlOH$_2$F$_x$

of 1 (S1) and 2 (S2), respectively (Figure 3a). Those signals' maximum intensity corresponds to fluorine concentrations of 4.2 and 7.4 mM for S1 and S2, respectively, at a pH value of 3 (data not shown). These intensities are almost constant for pH ≤ 4, and rapidly decrease at higher pH until they almost disappear at pH 6 (S1) and pH 7 (S2) (Figure 3b). Furthermore, the line width of S2, roughly similar to that of S1 at room temperature, broadened with decreasing temperature (at 276 K, the line width ratio $W_{S2}/W_{S1} \approx 2$).

Resonances S3 and S4. These two signals show a very similar fluorine concentration dependence. They are observed over a large pH scale (from 1 to 8 for S3 and from 3 to 8 for S4), and a broad range of [F]/[Al] ratios (from 2 to 8, Figure 3). The [F]/[Al] dependence is roughly bell shaped with a flat maximum occurring for [F]/[Al] values approximately between 4 and 6. Their highest maximal intensity is found to be about 19 mM (pH 2) and 10 mM (pH 7) for S3 and S4, respectively. Moreover, an accurate measurement of the signal S3 intensity at pH 5 shows a shoulder corresponding to [F]/[Al] = 3 (Figure 3d). This shoulder is seen at every acid pH but is not very pronounced at pH 3. Such a shoulder is not detected for signal S4.

Another difference between these two resonances is the dependence of their maximum intensities (I_{S3} and I_{S4}) on pH: I_{S3} decreases as the pH increases, whereas I_{S4} increases. Under these conditions, for values of pH ≥ 7 the ratio $[I_{S4}]/[I_{S3}]$ becomes greater than 1. Furthermore, varying the temperature from 276 to 323 K results in the coalescence of signals S3 and S4 (Figure 4), with a corresponding exchange temperature of about 316 K.[19]

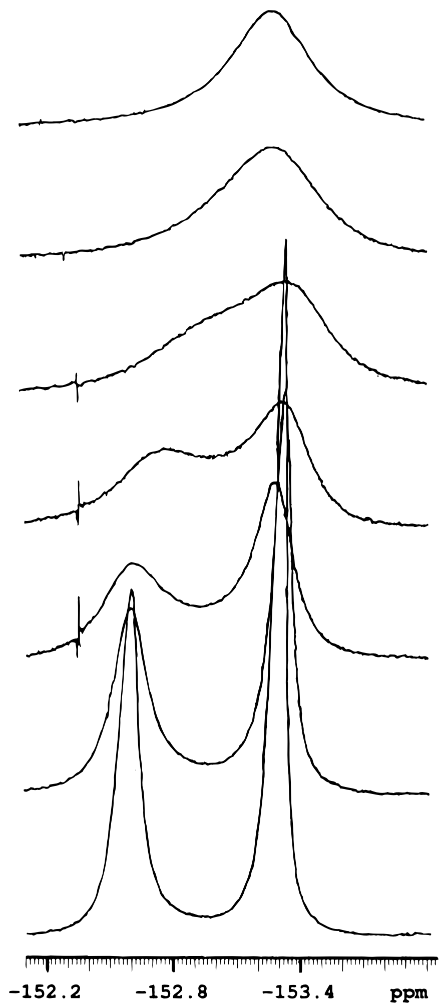

Figure 4. Temperature dependence of the ^{19}F NMR (470.26 MHz) spectra of solutions containing 5 mM Al(NO$_3$)$_3$ and 20 mM NaF, pH = 6.7. The coalescence temperature of the signals S3 and S4 is about 316 K.

Resonance S5. This signal is only observed at pH = 8 for [F]/[Al] ratios ranging from 3 to 5 (Figure 3c) and is of low intensity.

Discussion

The aluminum nucleus has a spin of $^5/_2$ and a relatively low quadrupolar moment ($Q_{Al} = 0.149 \times 10^{-28}$ m^2). As a result, aluminum NMR is characterized by a signal to noise ratio high enough to permit studies, such as reported here, at relatively low concentration. In addition, variations in the line width can be monitored, since the line width is low when the nucleus is in a perfect cubic geometry (*i.e.* octahedral or tetrahedral). The line width increases when the geometry of the nucleus diverges from cubic symmetry and can become so large that the signal merges into the background noise. In all cases, the total mass in the external reference permits a quantitative estimation of the mass of aluminum corresponding to the observed resonance. An extensive study of the chemical shifts of ^{27}Al NMR derivatives[15] showed that the coordination degree of the aluminum ion is directly correlated to the value of the chemical shift. For aluminum halides, resonance signals located between −40 and 20 ppm from Al(H$_2$O)$_6$$^{3+}$ correspond to hexacoordinate species (in the octahedral geometry), while tetracoordinate

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species (in the tetrahedral geometry) give rise to low-field signals located between 60 and 110 ppm.[20]

In the limit of our detection range, we have not noticed any ^{27}Al NMR signal in the 60/110 ppm region. A tetracoordinated AlF_4^- complex present in the solution, being highly symmetrical, would give rise to a narrow ^{27}Al resonance line in the appropriate frequency domain. No such signal was observed during the experiments reported. Coupling patterns have previously been observed[17] for AlF_6^{3+} under particular conditions (90% H_2O_2 and 10% H_2O) with a coupling constant $^1J_{Al-F}$ of 19 Hz. If we assume similar coupling constants in aqueous solution, the absence of coupling patterns may be explained by fast intramolecular motions between the aluminum ligands.

It has been observed[13] that, as long as the coordination is the same and the other ions bound to aluminum are similar, the chemical shifts of the ^{19}F NMR resonances corresponding to the various fluoroaluminate complexes move progressively downfield as more additional fluorine atoms are complexed. Hence, a more shielded resonance should correspond to a less fluorinated complex. The stoichiometry of complexes **1** and **2** can be obtained from the [F]/[Al] ratio for which the fluorine signal is at a maximum, derived from the calibrated intensities of the fluorine resonances. This fluorine concentration value for the S1 resonance represents almost all the fluorine present in the solution, when [F]/[Al] = 1. A similar observation applies for complex **2**, when [F]/[Al] = 2. Furthermore, the decreasing line width of the S2 resonance with increasing temperature suggests that chemical exchange processes may contribute to this signal. All these observations lead us to assign the S1 resonance to the complex $AlF_1(H_2O)_5^{2+}$ and the S2 resonance to the complex $AlF_2(H_2O)_4^+$. The chemical shift range observed by ^{27}Al NMR indicates an hexacoordinated geometry. These assignments are in accordance with previous results.[9] The broadening of the S2 resonance at low temperature can be explained as an exchange between the two possible *cis* and *trans* isomers.

The other ^{19}F NMR signals, corresponding to lower field resonances, cannot be unambiguously assigned to the expected fluoroaluminate complexes containing three, four, five, or six fluorine nuclei per aluminum. Previous studies[9] assigned the resonances S3 and S4 to the complexes AlF_3 and AlF_4^-, respectively. These assumptions took into account neither the pH dependence of the intensities of the S3 and S4 resonances nor the stoichiometry of the number of fluorine nuclei involved in the maximum signal intensity. In particular, the pH of the medium at which the spectra were taken is not specified (our data permits us to assume this pH was about 3). Our following assignments are not in agreement with these attributions.

At pH 2, the concentration of fluorine related to the S3 resonance has a maximum value of 19 mM, for a 5 mM concentration of aluminum. Thus, it means that *at least* four fluorines per aluminum are involved in the complex which is responsible for this signal. On the other hand, the shoulder observed at almost every studied pH corresponding to [F]/[Al] = 3 leads us to suspect the presence of a species possessing three fluorine ligands. Therefore, we propose that this signal is probably due to the coalescence of the signals of $AlF_x(H_2O)_{6-x}{}^{(3-x)+}$ complexes, in which x ranges from 3 to 4 and possibly up to 6. This assumption is strengthened by the large [F]/[Al] range in the existence of its fluorine resonance. This exchange, fast on the ^{19}F NMR time scale, could be related to the disappearance of ^{19}F and ^{27}Al NMR signals for the highest fluorinated species. This has been already described in highly fluorinated species[16] as arising from a fast exchange between free and bound fluoride. Signal S4, observed over a broad range of fluorine concentration and approximately concomitant with the observation of signal S3, probably results from the coalescence of the resonances from several chemical species. This will also contribute to the disappearance of the NMR signals at high [F]/[Al] ratios. On the other hand, a more likely explanation for the behavior of the pH dependent ratio $[I_{S4}]/[I_{S3}]$ is that S4 arises from complexes having the same degree of fluorination as S3, but where one OH^- replaces one H_2O ligand on the aluminum. This assumption is strengthened by the temperature dependent exchange between S3 and S4. Furthermore, S4 is not seen until [F]/[Al] = 3 and may not then correspond to species with less than two fluorines. Therefore, S4 can be assigned to the hexacoordinated complexes $AlF_xOH(H_2O)_{5-x}{}^{(2-x)+}$ with x ranging from 3 to 4 and possibly up to 5.

The assumption presently proposed of a single resonance arising from two or more species observed under fast exchange conditions and without any significant ^{19}F chemical shift dependence on [F]/[Al] can only be explained if we suppose that the species present have very similar chemical shifts. This assumption is in contradiction with the observation of the progressive deshielding of fluorine in fluoroaluminates. However, a similar absence of chemical shift dependence has already been observed[22] with ^{29}Si NMR on halide derivatives in the same geometry. On the other hand, the poor resolution of ^{27}Al spectra does not permit a discrimination between the different species. Even if the assignment of these two signals (S3 and S4) seems questionable, as deduced from the very small changes in ^{19}F chemical shifts between all these complexes and from the ^{27}Al NMR, the geometry of all these complexes still corresponds to an hexacoordination. One could assume that if the geometry of the complexes changed from octahedral to tetrahedral, then noticeable shielding shifts would result, as has been observed for two fluoroaluminate anions[21] and SiF_x complexes[22] (between 30 and 40 ppm). Furthermore, several runs with shift range extended to 50 ppm upfield did not show any additional resonances.

Signal S5, which is always of low intensity compared to the others, appears only at pH 8 and may correspond to a species of higher hydroxylation state of aluminum than the S4 species, *i.e.* two hydroxyl ligands per aluminum. Like S3 and S4, S5 is observed only for [F]/[Al] ratios higher than 2, thus indicating at least three fluorines per aluminum, and may also be considered as a coalescence of different resonances. As the ^{19}F NMR signal chemical shift is in the same range as the others, the species in question should have an octahedral geometry and thus leads us to assign this signal to $AlF_x(OH)_2(H_2O)_{(4-x)}{}^{(1-x)+}$ complexes with x = 3 or 4.

The equilibrium constant $K_{AlF_{i+1}}$ corresponding to the equilibrium

$$[AlF_i(H_2O)_{6-i}]^{(3-i)+} + F^- \leftrightarrow [AlF_{i+1}(H_2O)_{5-i}]^{(2-i)+} + H_2O$$

can be estimated for the first terms (i = 0, 1, 2) from the concentration dependence curves measured with the ^{19}F NMR data. Since the more these curves differ from each other, the more accurate the result, K_{AlF_3} can only be coarsely estimated, its dependence curve merging with the ones corresponding to the higher fluorinated complexes. This estimation was calcu-

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lated at pH 3 with a TOT simulation program[24] and gave the following values: $pK_{AlF_1} = 6.3$, $pK_{AlF_2} = 4.8$, and $pK_{AlF_3} = 3.3$, which are in agreement with the litterature[12] (6.4, 5.2, and 3.9, respectively).

Conclusion

The conditions for which fluoroaluminate complexes are able to interact either with G-proteins or with ATPases (in the presence as well as in the absence of ADP) correspond to [F]/[Al] ratios of 3−4. Previous results lead to the conclusion that AlF_3, AlF_3OH, or AlF_4 could be present in the active site of the protein.[25] Additional assumptions concerning the structure of the fluoroaluminate complexes suggest a tetracoordinated state for AlF_4. Our present results are not in agreement with this conclusion: (i) the ^{27}Al NMR spectra of fluoroaluminates in aqueous solution, at pH's lower than 8, never show observable resonances in the range of chemical shifts corresponding to the tetracoordinated species, (ii) the ^{19}F spectra are characterized by coalescence of resonance signals due to fast exchange conditions, which in turn imply that the species concerned are not undergoing major structural rearrangements, and (iii) there is no signal 30/40 ppm upfield which should correspond to tetrahedral species.[21,23] These results permit the conclusion that the octahedral nature of the coordination sphere is predominant for fluoroaluminate complexes in aqueous solution, for all pH's less than 8, and for all [F]/[Al] ratios. This conclusion helps to explain the octahedral geometry found for fluoroaluminate complexes bound to GDP either inside the active site of crystalline α-transducin[11] or inside the active site of the G-protein Giα1.[10]

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Supporting Information Available: Figures corresponding to intensities of ^{19}F signals *vs* the fluorine/aluminum ratio at pH 2, pH 3, pH 5, and pH 6 (4 pages). Ordering information is given on any current masthead page.

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