The Existence of AlF_4^- in Aqueous Solution and its Relevance to Phosphorylase Reactions

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Abstract

The discovery that trace amounts of aluminium are necessary for fluoride to exert its biological influence has lead to numerous investigations into the nature of the active fluoroaluminate complex. Many workers have postulated AlF_4^- to be this species. In this study we show that in the presence of inorganic phosphate or ADP, fluoride is not able to complex aluminium(III), but rather the species $[Al(PO_4)_2H]^{2-}$ and $[Al(ADP)OH]^-$ are formed. In the absence of competing ligands, ^{27}Al NMR reveals that octahedral, hydrated aluminium fluoride complexes are formed, the most likely one being $[AlF_4(H_2O)_2]^-$.

Introduction

Since the discovery that fluoride is able to stabilize glucose 6-phosphatase [1, 2], and to influence the activity of adenylate cyclase systems [3] and of phosphodiesterase systems of retinal rod outer segments [4], it has been shown that trace amounts of Al^{3+} are necessary for these effects to be realised [5–7]. Lange *et al.* have shown that all the interactions of fluoride with microsomal phosphohydrolase are dependent on the presence of Al^{3+} ions [6]. Invariably the reported similarity between AlF_4^- and PO_4^{3-} in terms of geometry and size has lead to the conclusion that, in solution, it is the AlF_4^- species which is responsible for the activity. In this study we wish to show that, under the conditions used in the various investigations, this species does not exist and hence cannot be responsible for the observed activity. Indeed, under no conditions does AlF_4^- exist in aqueous solution. Rather we demonstrate that in the presence of coordinating phosphate (inorganic phosphate or nucleotide diphosphate) the species $[Al(PO_4)_2H]^{2-}$ and $[Al(ADP)OH]^-$ predominate. Since aluminium(III) binding to nucleotides is via the phosphate groups and not the purine or pyrimidine bases, we have used ADP and ATP to represent nucleotide di- and triphosphates in general. AMP could not be included in this investigation as the aluminium complex precipitates above pH 3 (unpublished results). In the absence of any competing ligand we show that the octahedral $[AlF_3(H_2O)_3]$ and $[AlF_4(H_2O)_2]^-$ species predominate.

TABLE I. Equilibrium Constants Used in Simulation Study $pAl^{3+} + qL^{n-} + rH^+ = [Al_pL_qH_r]^{3p-qn+qr}$

Complex	Log β	Complex	Log β
$[Al(OH)]^{2-}$	−4.97[b]	$[Al(PO_4)H]^+$	23.25[d]
HF	2.9[c]	$[Al(PO_4)H_2]^{2+}$	26.18[d]
$[AlF]^{2+}$	6.11[c]	$[Al(PO_4)_2H]^{2-}$	37.95[d]
$[AlF_2]^+$	11.12[c]	$HADP^{2-}$	6.08[e]
$[AlF_3]$	15.0[c]	H_2ADP^-	9.89[e]
$[AlF_4]^-$	18.0[c]	[Al(ADP)]	10.03[e]
$[AlF_5]^{2-}$	19.4[c]	$[Al(ADP)OH]^-$	4.18[e]
$[AlF_6]^{3+}$	19.8[c]	$HATP^{3-}$	6.24[e]
HPO_4^{2-}	11.81[d]	H_2ATP^{2-}	9.94[e]
$H_2PO_4^-$	18.31[d]	$[Al(ATP)]^-$	8.96[e]
H_3PO_4	19.51[d]	[Al(ATP)H]	12.47[e]

[a]For simplicity, the coordinated solvent has been omitted. [b]Ref. 11. [c]Ref. 12. [d]Ref. 10. [e]Ref. 9.

Experimental

Computer simulation studies were carried out using the published program ESTA1 [8] and the conditions given in the legend to the figures. ^{27}Al and ^{19}F NMR spectra were recorded in 5 mm sample tubes on an NMC-250 spectrometer. $Al(NO_3)_3$ and NaF (Sigma Chemical Co.) were used without further purification. The constants used in the simulation studies are given in Table I.

Results and Discussion

Computer Simulation

Figure 1a shows the effect of varying pH upon the species distribution of the aluminium fluoride system. Clearly the AlF_3 and AlF_4^- species predominate over much of the pH range. Only at high pH values does any appreciable hydrolysis of the

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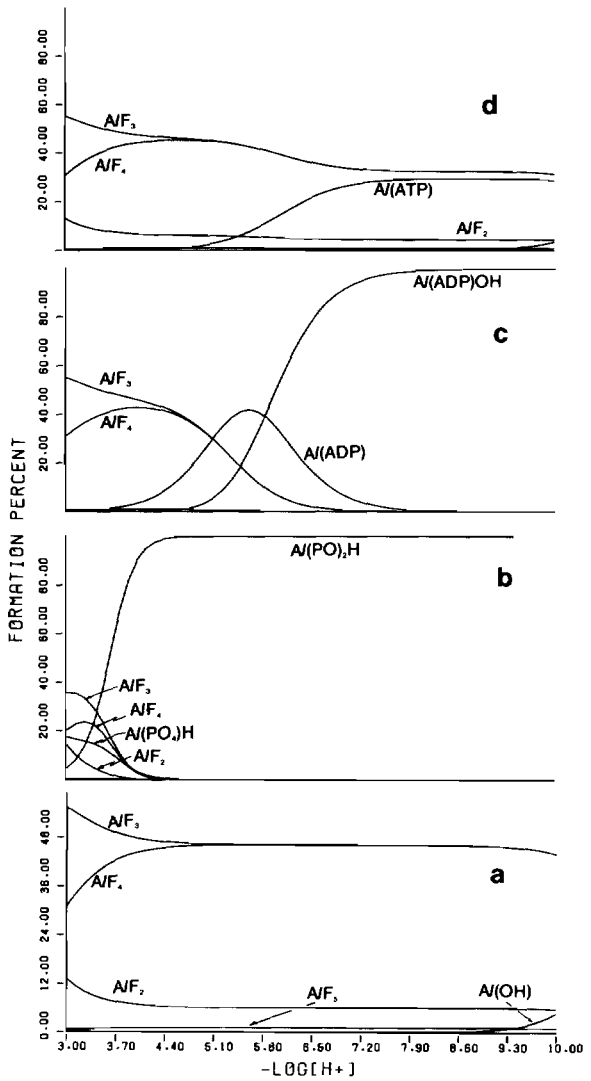

Fig. 1. Effect of pH upon the species distribution of 10^{-7} M Al^{3+} and (a) 1 mM F^-; (b) 1 mM F^-, 1 mM PO_4^{3-}; (c) 1 mM F^-, 1 mM ADP; (d) 1 mM F^- and 1 mM ATP.

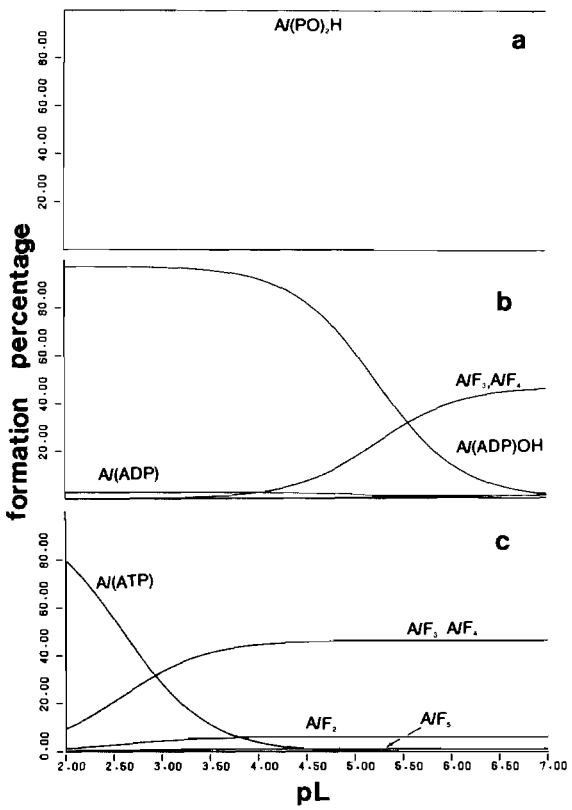

Fig. 2. Effect of varying ligand concentration upon the species distribution of 10^{-7} M Al^{3+} and 1 mM F^- at pH 7.4: (a) PO_4^{3-}; (b) ADP; (c) ATP.

aluminium(III) occur. However, this is only true in the absence of competing ligands. Recently we have investigated the aluminium/PO_4^{3-}/ADP/ATP systems [9, 10]. If any of these three ligands are introduced into the system a completely different picture obtains. For the Al^{3+}/ADP/F^- ternary system, between the pH values 9.4 and 7.4 virtually all the aluminium is complexed to ADP in the $[Al(ADP)OH]^-$ species. It is only below pH 6.4 that protonation of ADP takes place and that significant amounts of AlF_3 and AlF_4^- are formed. The case of ATP is very similar to that of F^- on its own, *i.e.* at no pH is ATP able to compete effectively against fluoride. The situation with inorganic phosphate, however, is completely different. It is not until pH 3.4 that an appreciable amount of aluminium fluoride complex is formed. Over almost the entire pH range only the $[Al(PO_4)_2H]^{2-}$ species exists in solution.

The above picture may not be strictly relevant to most biological studies which are normally conducted at a fixed pH of approximately 7.4. If this is the case then, assuming a fluoride concentration of 1.0 mM, by varying the concentration of the competing ligand, Fig. 2 is obtained. This again illustrates how effectively inorganic phosphate and ADP are able to compete with fluoride ions for aluminium(III). With inorganic phosphate, above a concentration of 10^{-7} M, 95% of the aluminium exists as the $[Al(PO_4)_2H]^{2-}$ species, while an ADP concentration of 10^{-5} M is needed to remove 60% of the aluminium from the fluoride. These simulation studies serve to illustrate that completely erroneous results can be obtained if the existence of competing ligands is ignored. Since the product of phosphorylase enzyme studies is invariably inorganic phosphate and a nucleotide diphosphate, their existence in solution cannot be ignored. If we assume equimolar concentrations of fluoride, inorganic phosphate, ADP and ATP, the species distribution diagram shown in Fig. 3 is obtained. Clearly, inhibition or stabilization cannot be occurring via the AlF_4^- species.

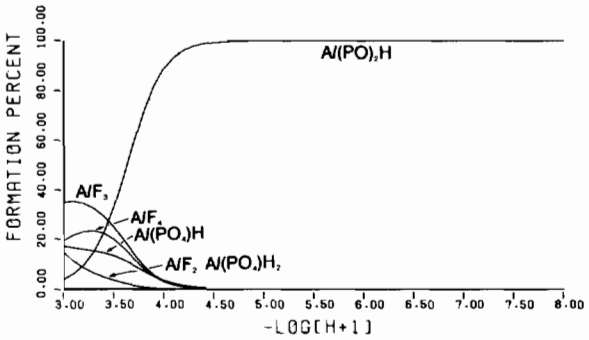

Fig. 3. Effect of pH upon the species distribution of a solution that is 10^{-7} M Al^{3+}, 1 mM F^-, 1 mM PO_4^{3-}, 1 mM ADP and 1 mM ATP.

While it is not strictly relevant to this study, some comment can be made about the effect of aluminium upon tooth decay. It is well known that during the formation of dental enamel dietary fluoride helps prevent formation of caries [13]. This occurs via the substitution of hydroxide by fluoride in the formation of hydroxyapatite.

$$Ca_5(PO_4)_3OH \xrightarrow{\text{demineralization}} 5Ca^{2+} + 3PO_4^{3-} + OH^- \xrightarrow{\text{remineralization}} Ca_5(PO_4)_3(OH)_{1-x}F_x$$

The fluorinated hydroxyapatite is then less susceptible to acid hydrolysis [14]. Aluminium is known to effect the formation of dental caries, but here both positive and negative effects have been found [15, 16]. The problem with aluminium is a multifaceted one. Aluminium may replace Ca^{2+} in tooth enamel and hence weaken the lattice structure [15, 17], in a way similar to the effect of aluminium on bone structure [18]. Secondly, by binding to phosphate, the saliva concentration of phosphate drops and hence demineralization is promoted. In addition, the high stability of the aluminium fluoro complexes may prevent fluoride remineralization. On the positive side, aluminium is known to inhibit certain enzymes and hence reduce the activity of dental plaque [19], one of the main causes of dental caries. Our simulation studies have shown that at the normal pH value of the mouth, 6.8, in the presence of either PO_4^{3-} or F^- there will be very little free aluminium with which remineralization can occur. On the other hand, the F^- ions will prevent the precipitation of aluminium hydroxide and hence retard plaque activity.

^{27}Al and ^{19}F NMR

One of the prime motivations for postulating the AlF_4^- species as the complex responsible for the

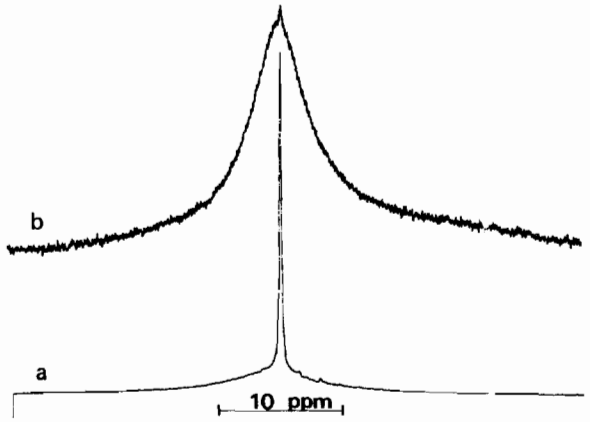

Fig. 4. The ^{27}Al NMR spectra of (a) 0.1 M Al^{3+}, 0.1 M F^-; (b) 0.1 M Al^{3+}, 0.2 M F^-. Chemical shifts are relative to $[Al(H_2O)_6]^{3+}$ as an external standard.

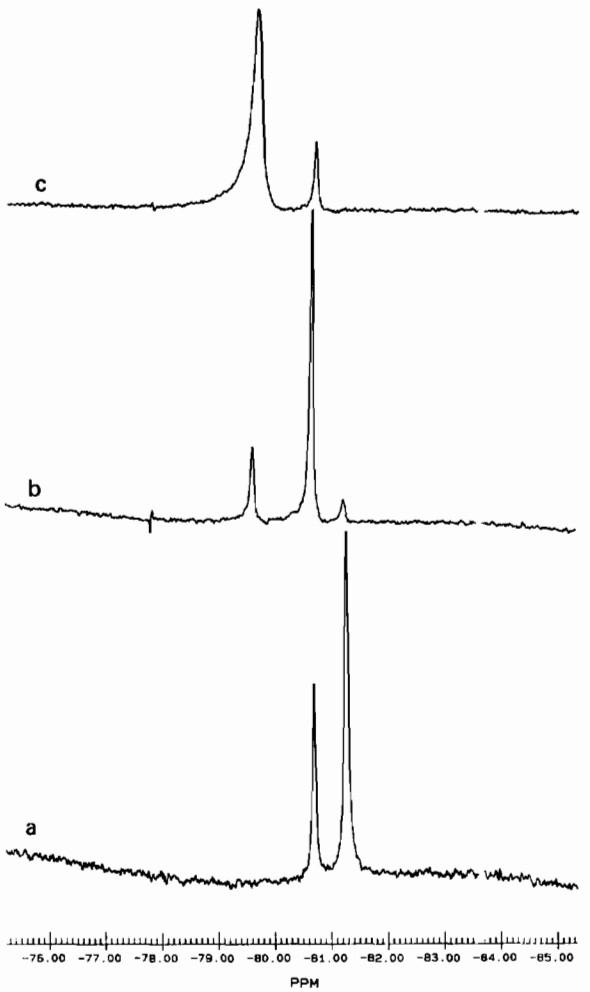

Fig. 5. The ^{19}F NMR spectra of (a) 50 mM $AlCl_3$, 50 mM NaF; (b) 33 mM $AlCl_3$, 67 mM NaF; (c) 25 mM $AlCl_3$, 75 mM NaF. Chemical shifts are relative to F^- as an external standard.

biological activity of aluminium fluoride solutions was the structural similarity between AlF_4^- and PO_4^{3-} [5]. Figure 4 shows the ^{27}Al NMR spectra of two solutions of aluminium at different concentrations of fluoride ion. The ^{27}Al chemical shifts of the various aluminium fluoride complexes are very similar, their difference being less than the line width, and hence they cannot be resolved. The large line widths of the various complexes is a clear indication that the Al(III) ion is experiencing a large electric field gradient and hence non-symmetrical, mixed complexes of the type $[AlF_n(H_2O)_q]^{+3-n}$ are being formed. If a tetrahedral AlF_4^- complex, rather than the octahedral $[AlF_4(H_2O)_2]^-$ complex were formed, a zero field gradient and hence a sharp ^{27}Al resonance would be obtained.

The ^{19}F NMR spectra of a series of aluminium fluoride solutions are given in Fig. 5. While the sequential formation of the different ALF_n species can be seen, no conclusions about the number of coordinated water molecules can be made. The existence of octahedral aluminium(III) in aqueous solution should not be surprising since even in the solid state NH_4AlF_4 contains octahedrally coordinated aluminium [20].

Conclusions

In this study we have attempted to show that the species AlF_4^- is not responsible for the biological activity of aluminium fluoride solutions because: (i) the species AlF_4^- does not exist in solution but rather the complex must be formulated as $[AlF_4(H_2O)_2]^-$; and (ii) under the conditions pertaining to most biological studies the aluminium is complexed to phosphate rather than fluoride. The problem still remains to explain the observed activity of aluminium and fluoride. We would suggest that it is the Al^{3+} ion which is responsible for the biological activity, possibly via coordination to phosphate. In the absence of added Al^{3+}, the F^- ion is necessary to solubilize Al^{3+} from the glass of the reaction vessel.

With regard to the diverse effects of aluminium on formation of dental caries, these can be explained in terms of concentration and time. In the short term, aluminium in low concentration as a mouth wash should decrease plaque activity and hence retard formation of caries [19], but at a high concentration and over an extended period, aluminium will become incorporated into the enamel structure and promote formation of dental caries [15, 17].

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