Aluminum: A Neurotoxic Product of Acid Rain

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Two separate but converging concerns have resulted in an upsurge in research on aluminum ion in the past 15 years. Acid rain releases Al(III) from soils into fresh waters, where it is for the first time accessible to living organisms. Though long considered benign, Al(III) has recently been found to cause bone and neurological disorders, while its role in Alzheimer's disease remains uncertain. The greater availability of Al(III), coupled with its demonstrated harmful effects, challenges chemists to describe its chemistry and biochemistry. Many interactions of Al(III) have been described, but several questions remain unsolved. A great deal of work not within the scope of this Account is described in several edited volumes.[1-4] (This Account uses Al(III) as a generic term for the 3+ ion when a specific form is not indicated.)

Occurrence

Comprising 8% of the earth's crust, aluminum is locked in minerals that conceal its status as the most abundant metal and the third most abundant element, after oxygen and silicon. In nature it occurs only in combined form: as an oxide in bauxite, the primary ore, and in complex aluminosilicates such as micas and feldspars. In contrast to its abundance in the crust, the ocean concentration is below 1 μg of Al/L; this low level may be the result of the accumulation of aluminum and silicon by diatoms. Until recently most natural waters contained insignificant amounts of aluminum, except for those in some volcanic regions and alum springs. Any freed Al^{3+} usually disappears into sediment as a hydroxide. With the advent of acid rain, metal ions such as aluminum, mercury, and lead escape from mineral deposits and dissolve in fresh waters. Acid rain serves as the key that springs the lock for metal ion release. The Al^{3+} concentration increases sharply in clear water lakes at pH <6 where micromolar amounts may occur.[5] Al^{3+} is more damaging to fish than increased acidity; even 5 μM Al^{3+} kills fish. Humans have set in motion an epic experiment to test the ability of living organisms to cope with higher levels of Al^{3+} activity. In the recent history of the earth, it is doubtful if the acidity of many natural waters has ever been higher.

Worldwide, given adequate nutrients, the presence of Al(III) is the main limiting factor in plant productivity in acidic soils. Plants such as tea that accumulate Al(III) do so in acidic soils and evidently detoxify the Al(III) by storing a chelated version in cell vacuoles of older leaves separate from the more metabolically active parts of the plant. Tea plants have been found with as much as 3% Al(III) in older leaves and only 0.01% in younger ones, a 300-fold difference.[6] Typical tea infusions contain about 50 times as much Al(III) as do infusions from coffee.[7] Adding milk to tea should immobilize Al(III) as an insoluble phosphate, while lemon will strongly complex the Al(III) in dangerous soluble citrate complexes described below.

Al(III) in Diseases

Coupled with the increasing availability of Al(III) is the role it plays in several human disorders. Al(III) is the likely cause of three conditions arising from long-term hemodialysis: vitamin D-resistant osteomalacia, iron adequate microcytic anemia, and dialysis dementia.[8,9] Because of the large amounts of dialysis fluid used, even small quantities of Al(III) may become deleterious over time. Dialysis centers now test for the Al(III) content. A recent study of 10^4 long-term hemodialysis patients finds that a serum Al(III) concentration of greater than 1.5 μM increases the mortality risk, and 40% of the patients exceed this level.[10] In normal individuals the serum Al(III) level is less than 0.4 μM. Similar conditions occur in children suffering from renal failure after prescribed high intakes of Al(III) to combine with excess phosphate of hyperphosphatemia. Upon acute ingestion even normal adults may accumulate Al(III) in bones.[11]

Al(III) is a possible cause of a high frequency of amyotrophic lateral sclerosis and Parkinsonism dementia among the natives of southern Guam, the Kii peninsula of Japan, and western New Guinea. In these areas the soils are high in Al(III) and low in Mg^{2+} and Ca^{2+}. Introduction of non-native diets has reduced the

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incidence of these ailments, which are associated with an accumulation of Al(III) in brain neurons that have undergone neurofibrillary degeneration into tangles, but without plaque formation.[12,13]

Elevated levels of Al(III) appear in the brains of Alzheimer's disease victims. About $^2/_3$ of cases of senile dementia are of the Alzheimer's type. There are about 4 000 000 sufferers of Alzheimer's disease in the United States. Despite the current search for genetic origins of diseases, fewer than 5% of Alzheimer's cases are familial. In the United States Alzheimer's disease afflicts ~3% of adults between 65 and 74, ~$^1/_6$ of those between 75 and 84, and ~$^1/_3$ of those greater than 85 years of age. Women are at greater risk. The disease ranks as one of the most frequent killers of the elderly, with 10^5 deaths annually in the United States. Although often not the direct cause of death, Alzheimer's disease causes enormous suffering among both victims and care givers. Alzheimer's disease is a progressive senile dementia characterized not by minor failures, such as the sufferer forgetting where she put her glasses, but rather by complete loss of input from a memory bank so she forgets that she wears glasses at all. There is no cure for the condition, which steadily worsens over many years. On a per case basis, Alzheimer's disease receives $^1/_{70}$ the research funding of the number with AIDS infections. Autopsies of Alzheimer's disease victims reveal deposition in the brain of fibrillar amyloid proteins as paired helical filaments that contain high levels of Al(III)[14] and of senile plaques that have been reported to contain aluminosilicate.[15] The presence of aluminum in senile plaques has recently been disputed.[16] However, the defining characteristic of Alzheimer's disease is neurofibrillary tangles with paired helical filaments. Whether Al(III) causes the paired helical filaments or possesses an affinity for the abnormal tangles remains uncertain.[17,18] Alzheimer's disease has been associated with the content of Al(III) in drinking water,[19] though the levels are still low compared to dietary intake. One of the unanswered questions is why elevated but still low levels of Al(III) in drinking water are associated with a higher incidence of Alzheimer's disease when the amount is still much less than that ingested daily in foods. This dilemma leads to questions of the significance of the association.[20]

Chronic exposure to large amounts of Al(III) in drinking water resulted in selective cognitive impairment in rats with altered calcium homeostasis, enhanced cyclic AMP production, and changes in cytoskeletal protein phosphorylation.[21] Combined with the demonstration that Al(III) causes dialysis dementia in humans, there seems little doubt that Al(III) is a dementing ion. Its association with Alzheimer's disease remains equivocal.

Table 1. Aluminum Intake by Healthy Adults

natural sources, about 2–5 mg/day
tea, typical 0.1% with up to 1% in dry tea, but poorly extracted in steeping, ~1 mg/cup
drinking water, average is 0.02 mg of Al/L
herbs, many high in Al but little consumed
spinach
food additives, about 10–20 mg/day
alum baking powder products, cornbread
emulsifier in American cheeses
in cream substitutes
aluminum silicates as anticaking agents
$Al_2(SO_4)_3$ as anticoagulant for turbid drinking water
in soy-based infant milk formulas
Al containers (new) in stewing of acidic fruits, spaghetti, sauce, sauerkraut (<0.1 mg/day)
pharmaceuticals, if used, to 4000 mg/day
antacids, 4000 mg/day for heavy users
buffered aspirin, 500 mg/day for arthritics
antiperspirants (avoid inhalation of spray)
vaccines (injection bypasses intestinal barrier)

Human Exposures to Aluminum

There are four avenues of exposure to nonessential aluminum among healthy adults. For an individual wide variations in intakes occur in each category, but some typical values appear in Table 1. Natural sources contribute only about 5 mg/day and probably even less for non tea drinkers. High aluminum levels occur naturally in only a few foods. Food additives add 5–100 mg/day with the high amounts due to alum baking powder products and Al(III)-containing emulsifiers added to American processed cheeses. Soy-based milk formulas provide a potential high Al(III) source to infants.[22] Except in instances of long stewing of acidic or highly salted foods, aluminum cookware, especially if not new, furnishes little aluminum to the diet.[23] As indicated by the last category in Table 1, the greatest potential sources of intake are Al(III)-containing antacids and buffered aspirins.[24] Ingested aluminum is poorly absorbed by the body. Most of what is absorbed is eliminated in the urine (probably in a citrate complex). The greatest danger from the use of antiperspirants may be spray inhalation and brain access via suggested nasal–olfactory pathways.[25] The last source in Table 1, vaccines, provides an example of solutions that bypass the intestinal barrier. Solutions given intravenously to patients may result in significant quantities of aluminum being injected directly into the blood and may be especially critical in infants.[26] Intakes by individuals with uremic problems,[27,28] industrial exposures, etc. are not covered in this list.

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Chemistry

To understand the roles of an element in an organism, we need to know not only the gross amount present but also the locale and complexes into which the element enters. The chemistry of aluminum is relatively simple. It reacts 10^7 times faster than Cr^{3+}, its hydroxide is much more soluble than that of Fe^{3+}, and it exhibits only one oxidation state in biological systems, Al^{3+}. Metallic Al is too reactive to be found free in nature, and the metal is won from its ores only with difficulty.[4,29] Thus there is no oxidation–reduction chemistry to Al^{3+} in biology.

We begin our survey of Al^{3+} by considering its ionic radius. In both mineralogy and biology comparable ionic radii are frequently more important than charge in determining behavior. The effective ionic radius of Al^{3+} in 6-fold coordination is 54 pm. By way of comparison, other values are Ga^{3+}, 62; Fe^{3+}, 65; Mg^{2+}, 72; Zn^{2+}, 74; Fe^{2+}, 78; and Ca^{2+}, 100 pm.[30] On the basis of the radii, though quite small, Al^{3+} is closest in size to Fe^{3+} and Mg^{2+}, and it is to these ions that we compare Al^{3+}. Ca^{2+} is much larger, and in its favored 8-fold coordination it exhibits a radius of 112 pm, yielding a volume 9 times greater than Al^{3+}. For this and other reasons we have argued that in biological systems Al^{3+} will be more competitive with Mg^{2+} than with Ca^{2+}.[29,31] Both Al^{3+} and Mg^{2+} favor oxygen donor ligands, especially phosphate groups.[32] Al^{3+} is 10^7 times more effective than Mg^{2+} in promoting polymerization of tubulin to microtubules.[33] In this study the free Al^{3+} concentration was controlled near 10^{-12} M with nitrilotriacetate (NTA). Also in plants, increasing Mg^{2+} reduces Al(III) toxicity.[34] Wherever there is a process involving Mg^{2+}, seek there an opportunity for interference by Al^{3+}.

Al(III), however, is not only a surrogate for Mg^{2+}. In erythrocytes Al(III) accelerates iron-stimulated lipid peroxidation by increasing membrane permeability.[35] Al(III) catalyzes covalent incorporation of phosphate into human τ protein.[36]

The most likely Al^{3+} binding sites are oxygen atoms, especially if they are negatively charged. Carboxylate, catecholate, and phosphate groups are the strongest Al^{3+} binders. Even when part of a potentail chelate ring, sulfhydryl groups do not bind Al^{3+}.[37] Amines do not bind Al^{3+} strongly except as part of multidentate ligand systems such as NTA and EDTA. Amino acids are weak binders barely competing with metal ion hydrolysis.[38] The nitrogenous bases of DNA and RNA do not bind Al^{3+} strongly.[29,39] The role of Al^{3+} in transamination reactions has been discussed.[40]

Exchange

In addition to stability of metal ion complexes, an important and often overlooked feature is the rate of ligand exchange out of the into the metal ion coordination sphere. Ligand exchange rates take on special importance for Al^{3+} because they are slow, and systems may not be at equilibrium. The rate for exchange of inner sphere water with solvent water is known for many metal ions, and the order of increasing rate constants in acidic solutions is given by

$$Al^{3+} \ll Fe^{3+} < Ga^{3+}, Be^{2+} \ll Mg^{2+} < Fe^{2+} < Zn^{2+} < Ca^{2+}$$

Each inequality sign indicates an approximate 10-fold increase in rate constant from 1.3 s^{-1} for Al^{3+} and increasing through 8 powers of 10 to 10^8 s^{-1} for Ca^{2+} at 25 °C.[38] Though these specific rate constants refer to water exchange in aquo metal ions, they also reflect relative rates of exchange of other ligands. Reducing Fe^{3+} to Fe^{2+} gains a 10^4-fold rate increase. Chelated ligands exchange more slowly, but the order remains. The slow ligand exchange rate for Al^{3+} makes it useless as a metal ion engaged in enzyme active site reactions. The 10^5 times faster rate for Mg^{2+} furnishes enough reason for Al^{3+} inhibition of enzymes with Mg^{2+} cofactors. Processes involving rapid Ca^{2+} exchange would be thwarted by substitution of the 10^8-fold slower Al^{3+}.

Al^{3+} Hydrolysis

Whatever ligands may be present, understanding the state of Al(III) in any aqueous system demands awareness of the species that Al(III) forms with the components of water at different pH values. In solutions more acid than pH <5, Al(III) exists as an octahedral hexahydrate, $Al(H_2O)_6^{3+}$, usually abbreviated as Al^{3+}. As a solution, becomes less acidic, $Al(H_2O)_6^{3+}$ undergoes successive deprotonations to yield $Al(OH)^{2+}$, $Al(OH)_2^{+}$, and a soluble $Al(OH)_3$, with a decreasing and variable number of water molecules.[29,41] Neutral solutions give an $Al(OH)_3$ precipitate that redissolves, owing to formation of tetrahedral aluminate, $Al(OH)_4^-$, the primary soluble Al(III) species at pH >6.2. Given time and the appropriate conditions, polynuclear hydroxo complexes may form. Several investigations[42,43] support formation of a soluble Al-13 polymer of composition $[AlO_4Al_{12}(OH)_{24}(OH_2)_{12}]^{7+}$ with a unique central tetrahedral Al^{3+} surrounded by 12 octahedral Al^{3+}.[44] This complex arises in diverse circumstances: it occurs in antiperspirants,[45] may be more phytotoxic than Al(III),[46,47] and is the pillaring agent in smectites.[48]

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The four successive deprotonations from $Al(H_2O)_6^{3+}$ to yield $Al(OH)_4^-$ squeeze into an unusually narrow pH range of less than 1 log unit with pK_a values of 5.5, 5.8, 6.0, and 6.2.[49] The narrow span for Al^{3+} is explained by the cooperative nature of the successive deprotonations due to a concomitant decrease in coordination number from 6 to 4.[41] While Al^{3+} is 3 log units less acidic than Fe^{3+}, $Al(OH)_4^-$ becomes the dominant species at almost 3 units lower pH than does $Fe(OH)_4^-$.[41] For Al^{3+} only two species dominate over the entire pH range, the octahedral hexahydrate $Al(H_2O)_6^{3+}$ at pH <5.5 and the tetrahedral $Al(OH)_4^-$ at pH >6.2, while there is a mixture of hydrolyzed species and coordination numbers in the range 5.5 < pH < 6.2 (distribution curves appear in the references).[4,38,41,50] (Slow exchange among complexes of differing coordination numbers may account for cases of multiple peaks in multinuclear NMR spectra of Al(III) complexes with several ligands including citrate and nucleotides.) If other ligands are incapable of holding Al(III) in solution, it becomes necessary to include the solubility equilibrium for $Al(OH)_3$.[29,38,41] To avoid precipitation, Al(III) may be administered internally to animals as a soluble citrate complex; use at least a 2:1 citrate to metal ion mole ratio. Maltol forms a weaker complex and permits a higher free Al^{3+} concentration.[38]

$$\mathbf{pAl = -log\ [Al^{3+}]}$$

In addition to the hydrolysis features already considered, the amount of free, aqueous Al^{3+} in solution depends upon several variables: ligands present, their stability constants with Al^{3+}, and total Al(III) to total ligand mole ratio. For ligands with protons competing with metal ion for binding sites in the pH range of interest, the pH is also a variable. Thus, instead of simple association of metal ion and basic ligand, $Al^{3+} + L \rightarrow AlL$, the relevant reaction may become displacement of a proton from the acidic ligand by the metal ion, $Al^{3+} + HL \rightarrow AlL + H^+$. For ligands containing amino, phenolate, and catecholate groups, the amount of free, aqueous Al^{3+} in neutral solutions becomes pH dependent. Thus, for these ligands, listed stability constants overstate effective binding strengths and need to be lowered to reflect competition of the proton with the metal ion for basic binding sites. The most practical method to allow for proton–metal ion competition at a ligand is to calculate *conditional* stability constants applicable to a single pH.[29,38,51] Conditional stability constants may also allow for deprotonation of metal ion coordinated water that yields more stable complexes with increasing pH in some Al^{3+} complexes, such as with citrate, nitrilotriacetate, and EDTA.

Results from quantitative evaluation of conditional stability constants are revealingly expressed as the negative logarithm of the free Al^{3+} concentration, $-\log [Al^{3+}] = pAl$. Analogous to pH, higher Al values represent lesser amounts of free Al^{3+}. Table 2 collects conditional stability constants and pAl values at pH 6.6 and 7.4 for several systems with 1 μM total Al(III) under the conditions indicated in the table. Weak Al^{3+} binders appear at the top and strong binders at the bottom of Table 2. The increasing pAl values as one goes down the table indicate decreasing free Al^{3+} concentrations. Thus, since 0.1 mM citrate lies lower in Table 2 then does 1 mM ATP^{4-}, we predict that citrate will withdraw Al^{3+} from ATP^{4-}, and experimentally citrate has been used for this purpose.[52] Despite high normal stability constants as indicated in the second column, salicylate[53] and catecholamines[54] bind Al^{3+} relatively weakly because competition from the proton in neutral solutions leads to the low conditional stability constants listed in the third and fifth columns. Kaolinite is the least soluble aluminum silicate.[55] Soluble aluminum silicates occur in fresh waters.[56] The strong Al^{3+} binder deferoxamine (last entry in Table 2) has been used to remove Al(III) from aluminum-intoxicated patients and has been reported to delay the progression of Alzheimer's disease.[57]

Table 2. Negative Logarithm of Free Al^{3+} Concentration, pAl[a]

complex or ligand[b]	log K_s	pH 6.6 log $K_{6.6}$	pH 6.6 pAl	pH 7.4 log $K_{7.4}$	pH 7.4 pAl
DNA	<5.6	<5.6	<7.3	<5.6	<7.3
salicylate, 0.2 mM	12.9, 10.6	6.3, 4.0	9.1	7.1, 4.8	10.7
amorphous $Al(OH)_3$	insoluble		9.1		11.5
Al^{3+} to $Al(OH)_4^-$	ref 41		9.1		12.1
catecholamines	15.6, 13.0	7.4, 4.8	9.7	9.0, 6.4	12.8
kaolinite[c]	insoluble		10.2		12.6
$AlPO_4$	insoluble		10.7[d]		11.4[e]
nitrilotriacetate (NTA)	11.1	10.0	11.7	11.6	13.3
2,3-DPG, 3 mM	12.5	11.6	12.2	12.2	13.1
ATP, 1 mM	7.9, 4.6	8.9	12.3	9.8	13.0
citrate, 0.1 mM; Ca^{2+}, 3 mM	ref 38	10.3	12.3	11.7	13.7
citrate, 0.1 mM	8.1	11.3	13.3	12.7	14.7
transferrin	ref 81			12.9, 12.3	14.6
F^-, 5 mM, with OH^-	ref 85		14.9		15.1
EDTA	16.2	13.1	14.8	14.7	16.4
deferoxamine	24.1	16.8	18.4	19.2	20.8

[a] 1 μM total Al(III) except for insoluble salts. Equilibria in addition to those related to listed log K_c values often required to calculate pAl. [b] 50 μM ligand unless otherwise noted. [c] $Al_2(OH)_4Si_2O_5$ with 5 μM $Si(OH)_4$, typical of plasma; see ref 55. [d] 10 mM total phosphate. [e] 1.1 mM total phosphate.

Phosphates

In the human body extracellular fluids contain about 1.1 mM total phosphate at pH 7.4 and intracellular fluids about 10 mM total phosphate at pH 6.6. Al^{3+} forms an insoluble salt with phosphate often designated as $AlPO_4$ but of more complex composition.[58] By analysis of this composite precipitate, we obtain the pAl values listed as the seventh entry in Table 2.

For the purposes of metal ion binding, soluble phosphate groups may usefully be divided into two classes: basic phosphates and weakly basic or nonbasic phosphates. Basic phosphates with pK_a = 6–7 are monosubstituted with a 2– charge and occur as $HOPO_3^{2-}$, as the terminal phosphate in nucleoside

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mono-, di-, and triphosphates, and in many other compounds. Weakly basic or nonbasic phosphates with the only $pK_a < 2$ are di(or tri)-substituted, bear a 1– charge, and appear as the internal phosphates in nucleoside di- and triphosphates and in DNA and RNA. Metal ions bind strongly to the basic phosphates and only weakly to the nonbasic phosphates. The disubstituted phosphates of the nucleotide polymers bear one negative charge per nucleotide residue, and the polymers behave as polyelectrolytes binding most metal ions weakly and nonspecifically. (Exceptions are specific loops in some RNAs and metal ion binding by the nucleic bases which are not considered in discussion of the phosphates.)

Al^{3+} binds strongly to basic phosphate groups. Some stability constants recently determined for Al^{3+} binding to 2,3-diphosphoglycerate (DPG)[59] and nucleotides[60] appear in Table 2. The strongest stability constants appear for ADP and ATP where chelation occurs. For comparison, the stability constant for Mg^{2+} binding to ATP and other nucleoside triphosphates is log K_1 = 4.3,[61] 4000 times weaker than for Al^{3+}. Thus, 0.2 μM Al^{3+} competes with 1 mM Mg^{2+} for ATP. (From interpolation in a linear log K_s vs pK_a plot for Al^{3+} binding to several monophosphates,[62] we estimate log K_s = 6.1 for the complexation reaction $Al^{3+} + HOPO_3^{2-} \rightarrow HOPO_3Al^+$. Even with subsequent deprotonations from a soluble complex,[50] the interaction is too weak to be competitive with other Al^{3+} interactions in biological media (Table 2).)

Many suppose that Al^{3+} binds to DNA in the cell nucleus. However, Al^{3+} binding to DNA is so weak that a quantitative study was limited to a high pH = 5.5 owing to metal ion hydrolysis and precipitation.[63] Therefore, DNA cannot compete with ATP and other ligands in Table 2 for Al^{3+}. Residing at the very top of Table 2 with the lowest pAl values or highest allowed free Al^{3+} concentrations. DNA loses Al^{3+} to all other entries in the table. We deduce that Al^{3+} binding to DNA is so weak under intracellular conditions that it fails by several orders of magnitude to compete with either metal ion hydrolysis or insolubility of even an amorphous $Al(OH)_3$. Therefore, we conclude that the observation of aluminum with nuclear chromatin is due not to its coordination to DNA but to other ligands containing basic phosphates.

What ligands might bind Al^{3+} in the cell, especially in the nuclear chromatin region? ATP and ADP are comparably strong Al^{3+} binders. A crucial Al^{3+} binding site in chromatin promises to be phosphorylated proteins, perhaps phosphorylated histones. Phosphorylation and dephosphorylation reactions normally accompany cellular processes. The phosphate groups of any phosphorylated protein provide the requisite basicity and, in conjunction with juxtaposed carboxylate or other phosphate groups, become strong Al^{3+} binding sites. Abnormally phosphorylated proteins have been found in Alzheimer's diseased brains.[64,65] Al^{3+} aggregates highly phosphorylated brain cytoskeletal proteins[66] and induces conformational changes in a phosphorylated neurofilament protein.[67] High Al(III) contents have been found associated with increased linker histones in the nuclear region of Alzheimer's diseased brains.[68] Very possibly Al^{3+} cross-links proteins, and proteins and nucleic acids.

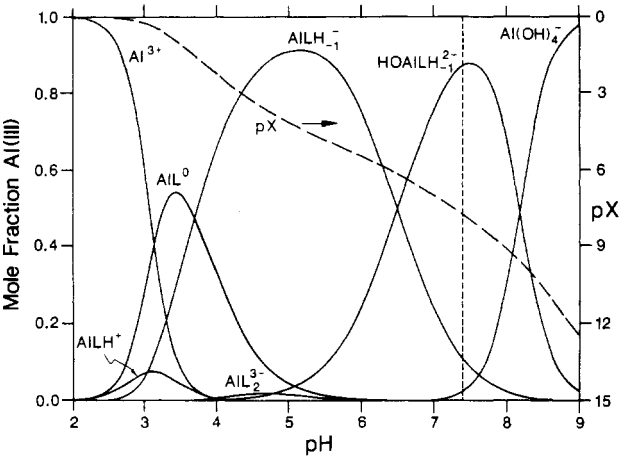

Figure 1. Mole fraction of Al(III) basis versus pH distribution curves (solids) for solution containing 0.1 mM citrate, 3 mM Ca^{2+}, and 1 μM total Al(III). The distribution is only very weakly dependent on the concentrations of the three components. The dashed line labeled pX refers to the scale on the right, where pX = –log(mole fraction of free Al^{3+}). Thus we have, at pH 7.4, pX = 7.7. Since we also have pAl = pX – log [Al(III)], for 1 μM total Al(III), pAl = 7.4 + 6.0 = 13.7 (in agreement with Table 2).

Citrate

It is as a citrate complex that Al(III) passes the intestinal barrier into the blood. Citrate exists mainly in the form of the tricarboxylate anion at pH > 6, and at 0.1 mM in the blood plasma it is the leading small molecule Al^{3+} binder.[39,69] In neutral solutions the main species is $HOAlLH_{-1}^{2-}$ followed by $AlLH_{-1}^-$ with $pK_a \simeq$ 6.5 for the loss of a proton from metal ion bound water.[29,49] Even though much of the citrate in plasma occurs as a Ca^{2+} complex, Al^{3+} easily displaces Ca^{2+} from citrate. Including consideration of alkaline earth cations in the plasma, there is an almost 10^8 mole ratio of citrate bound to unbound Al^{3+}.[38] Figure 1 shows the distribution curves for Al(III) species in the presence of plasma concentrations of 0.1 mM citrate and 3 mM total Ca^{2+} and Mg^{2+}. There is little difference between the distribution in Figure 1 and others derived without consideration of alkaline earth binding.[29,38,49]

As indicated in Table 2 and elsewhere,[29,38,39,58] citrate solubilizes Al^{3+} from both insoluble $Al(OH)_3$ and $AlPO_4$, making the metal ion more available to an organism. Proton nuclear magnetic resonance spectroscopy provides direct evidence that Al^{3+} binds to citrate in human

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blood plasma.[70] On the basis of information from equilibrium constants, it was strongly urged in 1986 that people should not take Al(III) and citrate together.[39,69]

There is ample proof that citrate facilitates incorporation of Al(III) into humans. Increased levels of serum Al(III) were found in patients with chronic renal failure who were taking an Al(III)-containing phosphate binder with citrate[71] or $Al(OH)_3$ with citrate.[72] A rapidly fatal encephalopathy in patients with chronic renal failure has been attributed to concomitant ingestion of $Al(OH)_3$ and citrate.[73,74] Moreover, healthy adults taking $Al(OH)_3$-based antacids along with citric acid, citrate salts, or citrus fruits showed substantial increases in Al(III) levels of blood[75,76] and urine.[77-80] The amount of citrate present should always be considered as a variable in Al(III) ingestion studies.

Transferrin

Transferrin binds two Al^{3+} and is the main protein carrier of Al^{3+} in the plasma. Displacement of the 10^9 times stronger binding Fe^{3+} is unnecessary because plasma transferrin is about 50 μM in unoccupied sites. The allowed pAl value for transferrin in blood appears near the bottom of Table 2, indicative of strong Al^{3+} binding by this protein.[81] On the basis of stability constants, we suggest that in human serum about 90% of Al(III) is bound to transferrin and 10% to citrate.[58] Cellular uptake of Al(III) occurs via binding of Al–transferrin at transferrin cell surface receptors.[82]

Catecholamines

In fluids low in citrate, transferrin, and nucleotides, the catecholamines may well become important Al^{3+} binders. While DOPA and epinephrine fail to bind Mg^{2+} at pH 7.4, they bind Al^{3+} at picomolar levels (Table 2). In neutral solutions the main species is a 3:1 complex with the catechol moiety chelating the Al^{3+} and the ammonium group remaining protonated.[51,54] The norepinephrine–Al^{3+} complex inhibits enzymatic O-methylation but not N-methylation by catechol *O*-methyltransferase.[83] This result conforms to that expected for Al^{3+} binding only to the catechol moiety of norepinephrine. When other metal ions are deficient,

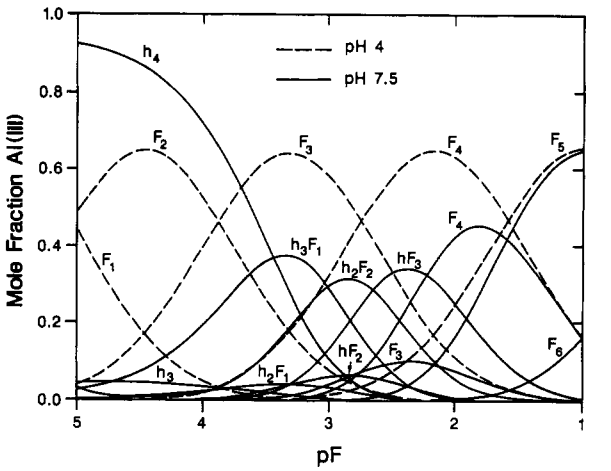

Figure 2. Mole fraction of total Al(III) versus pF = –log [F^-], where [F^-] is the ambient fluoride molar concentration, for fluoride complexes of aluminum at two pH values, dashed curves for pH 4 and solid curves for pH 7.5. Symbols on curves designate number of fluoride (F) or hydroxy groups (h) bound to Al(III). Thus h_4 represents $Al(OH)_4^-$; F_4, AlF_4^-; and hF_3, $(HO)AlF_3^-$. From ref 50, p 105.

Al(III) decreases catecholamine levels in the rat brain.[84] By binding to the catechol moiety of catecholamines, trace amounts of Al(III) may disrupt neurochemical processes.

Fluoride

We have also been investigating the stability of Al–fluoride complexes, their ternary complexes with hydroxide, and their mixed complexes with nucleotides. The composition of the ternary complexes with hydroxide depends upon the pH and the analogous pF = –log [F^-]. Figure 2 shows some of the results in the ternary system with hydroxide and is not as complicated as might appear at first sight.

Figure 2 displays distribution curves for mole fraction on a total Al(III) basis versus pF, which refers to the free, ambient fluoride molar concentration. The dashed curves for pH 4 illustrate the classic picture of successive Al–fluoride complexes. They are, however, valid only over a limited pH range. At pH < 4 the weak acid HF begins to form (pK_a = 3.0), and at pH > 5 Al^{3+} hydrolysis and ternary complexes with hydroxide start to appear. Many investigators have added Al–fluoride mixtures to systems at pH 7.5.

The solid curves in Figure 2 show the distribution curves at pH 7.5, and the greater number of species is evident.[50,85] The main starting species on the left at high pF (low ambient F^-) is the tetrahedral complex $Al(OH)_4^-$ (labeled h_4, where the lower case h represents a hydroxide group). Addition of fluoride successively replaces hydroxide groups to give as the predominant species $(HO)_3AlF^-$, $(HO)_2AlF_2^-$, $(HO)AlF_3^-$, and AlF_4^-, which is no longer tetrahedral. (The respective labels in the figure are h_3F_1, h_2F_2, hF_3, and F_4.) Further addition of fluoride results in the substitution of coordinated water molecules about hexacoordinate Al(III) to yield AlF_5^{2-} and finally AlF_6^{3-} just as at lower pH. Owing to intervention by bound hydroxide, at the

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higher pH, a greater fluoride concentration is required to obtain the same number of bound fluorides. Hexacoordinate F^- and tetrahedral OH^- are typical of third-row elements; consider the additional pairs: SiF_6^{2-} and $Si(OH)_4$, PF_6 and PO_4^{3-}, and SF_6 and SO_4^{2-}.

At the 1 ppm level of F^- addition to drinking water, pF = 4.3, and according to Figure 2 the main Al(III)-containing species becomes AlF_2^+ in acidic solutions and $Al(OH)_4^-$ at pH 7.5. A four times greater F^- concentration has been reported to protect against dementia.[86] Fluoride does not enhance leaching of Al(III) from cooking utensils.[87]

Investigators have added Al(III) and excess fluoride to protein systems such as G-proteins and have observed a peak effect at about 5 mM added fluoride. Inspection of the classic dashed curves in the figure reveals AlF_4^- as the dominant species at the corresponding pF = 2.3, and the results have been interpreted as showing this species serving as a tetrahedral pseudophosphate. However, in aqueous solution the AlF_4^- complex should be hexacoordinate with two bound waters. Moreover, most such experiments were performed near pH 7.5, where the match with the solid curves in the figure suggests the predominant species to be $(OH)AlF_3^-$ with three fluorides.

In experiments with G-proteins, the presumed tetrahedral pseudophosphate AlF_4^- is postulated to reside adjacent to GDP on the G-protein.[88] We have employed multinuclear NMR spectroscopy to study the ternary system Al(III), F^-, and nucleoside diphosphates (NDP) in aqueous solutions without protein.[89] Under a wide variety of conditions species with compositions (NDP)-AlF_x with x = 0–3 have been characterized; no species with x = 4 was detected. All of these complexes should have hexacoordinate Al(III). Under the conditions of the G-protein experiments at pH 7.5 with up to 0.1 mM GDP and a maximum effect at about 5 mM F^-, most Al(III) occurs as $(HO)AlF_3^-$; the lesser amount of nucleoside diphosphate is not competitive. Thus, under these conditions, the distribution curves in Figure 2 apply even to solutions with added nucleotides. This result is confirmed by the high pAl values for the F^- solutions in Table 2. Therefore, the proposed G-protein bound ternary complex $(NDP)AlF_4^{4-}$ does not occur to a significant extent in aqueous solution, where the presumed tetrahedral AlF_4^- is hexacoordinate with two bound water molecules. Recent work with transducin interprets $(OH)AlF_3^-$ as the relevant species.[90]

Concluding Remarks

As illustrated in this Account, chemistry has contributed importantly to the ramifications of increased aluminum in fresh waters and to its speciation and role in disease, especially those brought on by more than minimal levels of Al(III) in dialysis fluids. However, more interactions of Al(III) require elaboration. Naturally occurring silicic acid[91] and fluoride[92] reduce the toxicity of Al(III) to fish. Fluoride also ameliorates Al(III) toxicity in plants such as wheat.[93] In humans, dissolved silicon limits gastrointestinal absorption of Al(III)[94] and fluoride in drinking water reduces the incidence of dementia.[95] Clearly, the concentrations of all significant components need to be considered. When fluoride is added to any system containing calcium, insoluble CaF_2 may form. In the presence of 10 mM F^- insoluble CaF_2 allows only about 3 μM Ca^{2+}.[29] The effect of body iron stores upon aluminum uptake remains uncertain. Iron also accumulates in neurofibrillary tangles.[14]

What insights might chemists contribute to the question of possible Al(III) involvement in Alzheimer's disease? Al(III) may activate some processes,[33] inhibit some enzymes, and cross-link macromolecules. Alzheimer's disease involves formation of neurofibrillary tangles composed of paired helical filaments. Multivalent cations aggregate neurofilaments.[96] The proteins involved in filament formation are phosphorylated, perhaps hyperphosphorylated. As indicated above, Al^{3+} binds strongly to basic phosphates. Therefore, on the basis of its chemistry, Al^{3+} is an excellent candidate to promote formation of the characteristic paired filaments associated with Alzheimer's disease. Are tangles and plaques the unfortunate result of the brain's overzealousness in immobilizing and detoxifying Al(III)? On the other hand, already made phosphorylated tangles provide an ideal chelating site for adventitious aluminum. Is the presence of aluminum a coincidence, a consequence, or a cause of the characteristic tangles of Alzheimer's disease?

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