ELSEVIER

Coordination Chemistry Reviews
149 (1996) 329–346

COORDINATION CHEMISTRY REVIEWS

Interaction of aluminium(III) with phosphate-binding sites: biological aspects and implications

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Received 6 March 1995

Contents

Abstract

The occurrence of abnormal levels of Al(III) connected with human pathologies and with encephalopathies experimentally induced in experimental animals is reviewed and critically evaluated in molecular terms. It is assumed that phosphate-bearing biomolecules are elected targets for the biochemical action of Al(III) and the reactivity of the metal centre towards

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both inorganic phosphates and nucleotides, diphosphoglycerate, phosphorylated proteins, phospholipids and *myo*-inositol–phosphates is thoroughly reviewed. All these data are evaluated in the light of the potential relevance of higher Al(III) levels to the etiopathology of dialysis dementia and of Alzheimer's disease.

Keywords: Al(III)–biophosphate complexes; Solution speciation, Al(III) toxicity

1. Introduction

The toxicity of Al(III) to mammalians is established beyond any reasonable doubt. Histopathological, clinical and epidemiological observations [1] have linked an abnormal uptake and accumulation of Al(III) to important human pathologies such as dialysis dementia (DD) [2], iron-adequate microcytic anaemia [3], bone diseases [4] and Alzheimer's disease (AD) [5]. Moreover, administration of Al(III) to experimental animals has unambiguously revealed a marked neurotoxic character (experimental encephalopathy, EE) of the metal centre [6]. The Al(III) taken in every day, ca. 1–10 mg from natural sources [7], is carefully excreted in humans thanks to an evolutionary well developed defensive mechanism, which leads to the accumulation of only approximately 1/1000 of the metal centre introduced via diverse routes [8], provided healthy gastro-intestinal and renal conditions are operating. Although prevention strategies are successfully reducing the occurrence of pathologies associated with Al(III) exposures, understanding the molecular bases of Al(III) toxicity is still an open question which scientists with very diverse backgrounds are attempting to answer.

In addition to the obvious relevance of the "aluminium question" to the human quality of life, a substantial literature calls attention to the possible relevance of the natural exposure to Al(III) to the process of cell ageing [9].

Considering that the evolution of life in a very aluminium-rich biosphere did not result in a known physiological role of Al(III) in any living organism, the issue of "aluminium biology" still remains a challenge to modern biochemistry and bioinorganic chemistry. In this connection, the concentration levels of aluminium in the human body should be fully appreciated. According to various authors, the metal levels in human tissues range from 35 to 40 mg [10] and the total amount of aluminium in a healthy human brain is estimated to be of the order of 1 mg [11]. Still, such minute amounts are of concern because significant alterations from these physiological values are associated with severe pathologies, such as dialysis dementia (10–15 mg) [12] iron-adequate microcytic anaemia and aluminium-overload related bone disorders (see above).

Evaluation of the vast literature dealing with the interaction of Al(III) with biological systems suggests cellular and molecular hypotheses for aluminium toxicity, some of which are listed in Table 1. Of course, this list has to be considered as only a partial collection of phenomenological data and of reasonable hypotheses which are ranked in a non-systematic form.

In the frame of all these data and hypotheses, a potential role of Al(III)–phosphate interaction is either obvious or possible and this forms the basis of this review paper.

Table 1
Data and hypotheses on the molecular bases of Al(III) toxicity

Data and hypothesis	Ref.
Al(III) competes effectively with Mg(II) and therefore inhibits Mg(II)-dependent enzymatic activities, interfering with the utilization of ATP (AD, DD, EE)	[13]
Al(III) destabilizes plasmatic membranes, thus contrasting cells homeostasis and acting as a "pick-lock" on the blood-brain barrier (AD, DD, EE)	[14–18]
Al(III) binds to nuclear chromatin and acts on the transcription of genetic information in susceptible neurons, possibly increasing the stability of linker histone–DNA adducts (AD, EE)	[6,19,20]
Al(III) stimulates an abnormal lysosome activity in susceptible neurons (DD), leading to cellular autolysis	[21–23]
Al(III) interferes with the physiology of VDAC channels in susceptible neurons, eventually producing cell death (AD, DD, EE)	[24,25]
Al(III) competes with Ca(II) in calcifying tissues to give $AlPO_4$ (Fracturing osteomalacia)	[1]
Al(III) interferes with enzymatic activities and with the secretion of neurotransmitters (AD, EE). The results in this connection are contradictory and the conclusions are controversial, probably owing to important differences in administration protocols	[23,26]
Al(III) interferes with the biochemistry of inositol phosphate, severely perturbing the cellular physiology of Ca(II) inside neurons (AD, DD, EE)	[28]
Al(III) promotes the hyperphosphorylation of normal neurofilaments, favouring Al(III)-promoted cross-linking of hyperphoshorylated neurofilaments and promoting neurofibrillary degeneration (AD, EE)	[23,27,29]
Al(III) interferes with the normal production of red blood cells, leading to iron-adequate microcytic anaemia. However, the possible complex articulation of this interference is still far from being defined	[3]
Al(III) interacts with β-amyloid in AD non-mature senile plaques, contributing to the formation of the final highly insoluble protein aggregates	[30]

It is generally believed that Al(III) has a great affinity for phosphate ligating sites, so that the interaction of naturally intaken and circulating Al(III) with phosphate-bearing biomolecules (PBB) is expected to be a major circumstance in aluminium biology. The aim of this paper is to review in a necessarily non-exhaustive way: (i) the basic chemistry of the Al(III)–PO_4^{3-}–H_2O system; (ii) the occurrence and physiology of the major PBB; (iii) the phenomenological observations of Al(III) accumulation in biological domains rich of PBB; and (iv) the bonding interaction of Al(III) with PBB as revealed by potentiometric and, in general, instrumental analysis. The expected biological implications of the interactions of Al(III) with PBB are also evaluated.

2. The basic chemistry of the Al(III)–PO_4^{3-}–H_2O system

$AlPO_4$ (orthophosphate) ($pK_{ps} = 19.1$ at 37 °C and 0. 15 M ionic strength) occurs in nature as a variety of known water-insoluble minerals [31]. Among other known salts, we mention pyrophosphate [$Al_4(P_2O_7)_3$] and metaphosphate [$Al(PO_3)_3$] [31]. ^{27}Al NMR solid-state data show that Al(III) is tetracoordinate in crystalline $AlPO_4$

[32]. Recent results of Öhman and Martin [33] have shown that the poorly soluble species, which may precipitate from neutral aqueous solutions, is a mixed phosphato–hydroxo complex, with continuously variable proportions of phosphate and hydroxide. Under serum conditions, for instance, its composition may be described as $Al(PO_4)_{0.2}(OH)_{2.4}$.

H_3PO_4 is a polyprotic acid. The anion PO_4^{3-} does not exist in aqueous solution at pH values lower than 12 ($pK_a = 1.9$, 6.7 and 11.7) [34]. At physiological pH values, HPO_4^{2-} is the dominating species, and is in fact the relevant phosphate form present inside cells and in circulating biological fluids at concentrations of ca. 10 and 2 mM, respectively [13].

For intracellular fluids (pH = 6.6), $-\log[Al(III)]$ is predicted to be 11.7 and for extracellular fluids (pH 7.4) to be 12.1 [13]. Remarkably, Öhman and Martin [33] strongly suggested that in blood serum ca. 11% of total Al(III) is bound to citrate ca. 89% is coordinated to transferrin and that no appreciable Al(III)–phosphate bonding occurs.

3. The occurrence and physiology of major phosphate-bearing biomolecules in cellular systems

The major phosphate-bearing biomolecules are ATP, membrane phospholipids and nucleic acids. All these biomolecules possess potential ligating sites to Al(III) and metal coordination of these biomolecules might introduce critical disfunctions in fundamental biological processes relevant to cell homeostasis and, consequently, for their overall healthy condition.

ATP is a universal "currency of free energy" in the biological systems in that all living organisms require a constant availability of free energy for performing mechanical work, active transport of molecules and ions and macromolecular synthesis from simple precursors [35]. ATP is a nucleotide formed by an adenine, a ribose and a triphosphate (Fig. 1) unit present in the active form as a complex with Mg(II) or

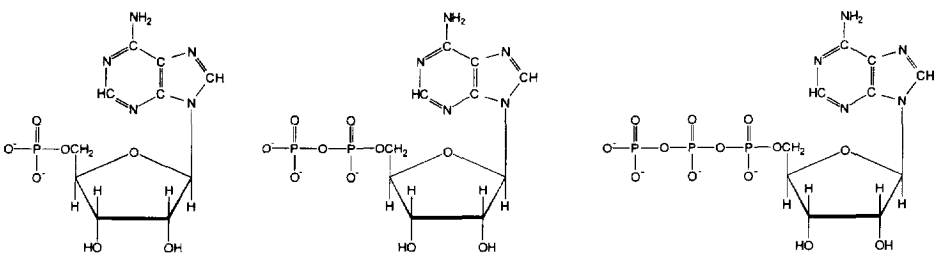

Fig. 1. Molecular structures of ATP, ADP and AMP.

Mn(II). The turnover of ATP is extremely high, a resting human consuming and restoring about 40 kg per day.

Various other phosphate-bearing biomolecules play roles in human physiology, i.e. adenosinediphosphate (ADP), adenosinemonophosphate (AMP), other nucleotides, 2,3-diphosphoglycerate, inositol triphosphate, etc. The last molecule is an important cell messenger and potential cytosolic ligand towards Al(III) [28].

4. The phenomenological observations of Al(III) accumulation in biological domains rich in PBB

Aluminium is found to occur in most human tissue, with the exception of the lung, at a very low (2–3 ppm, dry weight) and substantially constant level [1]. As already pointed out in Section 1, the total amount of Al(III) in a healthy normal human body is ca. 35–40 mg, but the metal level increases dramatically in the brain tissue of people suffering from renal failure and focal accumulation of Al(III) has been observed in phagolysosomes of neurons from a subject suffering from senile dementia [21]. In AD-affected patients, a moderate elevation of aluminium levels has been discovered by independent groups [5] and focal metal accumulation was identified by Perl and Brody [36] in the tangle-bearing neurons affected by neurofibrillary degeneration. In addition, Candy et al. [37] found Al(III) accumulation in the core of mature senile plaques separated from AD brains. Fine biochemical work performed by McLachlan and co-workers [20] in Toronto has shown that dinucleosome preparations from the brains of AD patients contain up to six times higher Al(III) levels compared with controls chosen among dementia-associated diseases including DD. On the basis of this finding, the most interesting and challenging localization of aluminium appears to the nuclear chromatin.

5. The bonding interaction of Al(III) with phosphate-bearing biomolecules

Al(III) shows a strong binding ability towards the PBB potential ligands. The reactivity of some PBB molecules (including DNA) with Al(III) has recently been reviewed by Martin [38]. On the basis of a strictly thermodynamic point of view, Martin evaluated log K_s for the Al(III)–DNA interaction under intracellular conditions to be equal to 5.6, i.e. more than 3.3 log units less than the conditional stability constant for ATP at pH 6.6. On the basis of the above, he predicted that in nuclear chromatin (in the presence of ATP) Al(III) should not be bound to DNA but to ATP or other comparable ligating sites such as those pending from phosphorylated proteins of linker histones. Incidentally, mutatis mutandis, on the basis of similar arguments the author predicted that nanomolar levels of Al(III) in contact with bone-forming domains will displace Ca(II) from phosphate binding and therefore will impair bone mineralization.

It is important to stress that Martin's arguments are strictly based on thermodynamics and relevant reasoning, which cannot be separated from kinetic circum-

stances, especially under experimental toxicological conditions. The biological relevance of kinetic inertness of Al(III) coordination chemistry has been stressed in [13,39] and the reluctance of the metal centre to modify its coordination sphere according to the thermodynamic requirement may well mitigate against predictions based thereon and introduce a further complication in defining the molecular bases of Al(III) toxicity.

We shall review in this section the information available on the reactivity of Al(III) with PBB (including inorganic phosphates) as evaluated from both potentiometric and other diverse analytical investigations.

For the purpose of metal ion binding capabilities, soluble phosphates may be divided into two classes: basic and weakly basic phosphates [38]. Basic phosphates with $pK = 6–7$ are monosubstituted, with the general formula $R–OPO_3^{2-}$, have a charge of 2− and occur as the terminal phosphate in nucleoside mono-, di- and triphosphates, phosphorylated proteins and also in many other biophosphates. Inorganic mono- and polyphosphates all contain at least one basic phosphate group. Weakly basic phosphates with a single $pK < 2$ are disubstituted with the general formula $R–O(R'–O)PO_2^-$, have a charge of 1− and occur as internal phosphates in nucleoside di- and triphosphates, in the nucleic acids DNA and RNA and also in phospholipids, phosphoglycolipids, etc.

5.1. *Al(III)–monophosphate*

The Al(III)–PO_4^{3-} equilibrium is difficult to study because of the formation of soluble polynuclear complexes, generally in slow processes and also the precipitation of poorly soluble species at higher metal ion concentration and at $pH > 4$. This is the main reason why the results of potentiometric speciation measurements are so scattered as they appear in Table 2. [In the tables, $\log K$ values always refer to the stepwise protonation processes of the ligands, while overall stability constants, $\beta_{pqr} = (M_pL_qH_r)/(M)^p(L)^q(H)^r$, refer to the Al(III) complexation steps.]

Therefore, the approach of linear free energy relationships (LFERs) was used in two studies in order to characterize the interaction between Al^{3+} and PO_4^{3-} in solution. Since complex formation in these cases is competition between Al(III) and H^+ for the ligand PO_4^{3-}, a close correlation can be expected between the protonation and the Al(III) complexation processes of the ligand, assuming a similar binding mode in the Al(III) complexes. Harris [40] constructed LFERs between the overall basicity of the coordinating donor groups of a series of O-donor ligands and the corresponding Al(III) binding constant. He found a well defined linear relationship between the overall basicity and the Al(III) binding affinity for bidentate ligands forming five-membered chelates. With the assumption that inorganic phosphate also coordinates as a bidentate ligand a phosphate binding constant $\log \beta_{AlPO_4} = 14.10$ and in a similar way another stability constant for the hydrolysed species $Al(PO_4)(OH)^-$, $\log \beta_{AlPO_4(OH)} = 8.37$ were determined. Atkári [41] constructed LFERs for a series of organic monophosphates (including mononucleotides) and phosphonates and obtained reliable stability constants for the monoprotonated soluble complex $Al(HPO_4)^+$ ($\log \beta_{AlHPO_4} = 17.40$) and the mixed hydroxo species

Table 2
Proton (log K) and aluminium(III) (log β) stability constants for the complexes of monophosphate (L^{3-})

Parameter	Ref. [41][a]	Ref. [42][b]	Ref. [43][c]	Ref. [44][b]	Ref. [45]	Ref. [40]
Log K_{H_3L}	1.86	1.96	1.2	2.0	–	–
Log K_{H_2L}	6.63	6.61	6.51	6.68	–	–
Log K_{HL}	11.48	11.39	11.80	11.54	–	–
$AlLH_2^{2+}$	19.65(6)	22.25(4)	26.18(1)	20.93(4)	<21.0	–
$AlLH^{+}$	17.40(3)	19.1(1)	23.25(1)	17.79(1)	<18.4	–
AlL	–	15.7(4)	–	15.32(5)	–	14.10
$AlLH_{-1}^{-}$	–	–	–	–	–	8.37
AlL_2H^{2}	–	–	37.95(10	–	–	–
Al_2L^{3+}	17.42(2)	20.9(1)	–	18.72(5)	–	–
$Al_2LH_{-2}^{+}$	11.05(2)	15.80(6)	–	12.58(5)	–	–
Al_2LH_{-3}	6.9(1)	6.7(2)	–	–	–	–
Log K^{*}[d]	5.92	7.7	11.45	6.25	–	–
pL_{AlPO_4}	–	19.1(1)	–	18.34(2)	–	–
$pL_{Al(OH)_2H_2PO_4}$	–	27.6	–	–	–	–

[a] $I=0.20$ M KCl, $t=25$ °C.
[b] $I=0.15$ M NaCl, $t=37$ °C.
[c] $I=0.15$ M NaCl, $t=25$ °C.
[d] Log K^{*} characteristic of the reaction $Al^{3+}+HPO_4^{2-}=Al(HPO_4)^{+}$.

$Al(HPO_4)(OH)$ and $Al(HPO_4)(OH)_2^{-}$ or $Al(PO_4)(OH)^{-}$ (log $\beta=14.4$ and 7.4, respectively). Besides these monomeric complexes, the potentiometric titration results suggested the formation of various dinuclear, partly mixed hydroxo species, such as $Al_2(PO_4)^{3+}$, $Al_2(PO_4)(OH)_2^{+}$ and $Al_2PO_4(OH)_3$ [42]. In these dinuclear complexes either phosphate and/or hydroxide can behave as bridging ligands. In a detailed ^{31}P and Raman spectroscopic study, Feng and Waki [46] observed the formation of a bis-complex also, at a tenfold excess of ligand; this species, however, could not be detected by any other method. However, the correct assignment of the NMR resonances is difficult. Unfortunately, no exact information is available on the actual stoichiometry of the species existing in the physiological pH range. This is why Al(III) speciation model calculations for biological fluids, such as serum, led to contradictory results [33,40,47,48]. In this connection, a recent paper by Öhman and Martin [33] reported inorganic phosphate to be too weak a binding molecule for Al(III) in plasma. Only hydroxide, as a second complexing ligand, was taken into account in these calculations and ternary complex formation with other ligands constituents of biological fluids was not considered.

5.2. *Al(III)–oligophosphates*

The metal binding ability of di- and other oligophosphates is much stronger than that of the monophosphate, owing to the formation of six-membered chelates via the adjacent phosphate groups [41,43,46]. Representative stability data reported for Al(III)–diphosphate (DP) and –triphosphate (TP) systems are listed in Table 3.

Table 3
Proton (log K) and aluminium(III) (log β) stability constants for the complexes of diphosphate (DP) and triphosphate (TP)

Parameter	DP		TP	
	Ref. [41][a]	Ref. [43][b]	Ref. [41][a]	Ref. [43][b]
Log K_{H_3L}	1.67	–	1.70	2.03
Log K_{H_2L}	5.87	6.34	5.34	5.64
Log K_{HL}	8.23	8.67	7.76	8.05
$AlLH_2$	18.69(3)	22.79	18.07(5)	–
AlLH	17.03(3)	19.20	16.65(4)	20.98
AlL	13.74(3)	14.30	13.15(4)	17.31
$AlLH_{-1}$	7.41(4)	–	6.53(5)	11.72
AlL_2H	25.64(3)	–	24.43(5)	–
AlL_2	19.77(1)	–	19.14(2)	–

[a] $I=0.2$ M (KCl) and $t=25$ °C.
[b] $I=0.15$ M (NaCl) and $t=25$ °C.

In contrast with monophosphate, oligophosphates readily form bis-chelated complexes, although the spatial requirement of the ligand molecules and also the electrostatic repulsion, due to the coordination of a highly charged second ligand molecule, is larger than in the case of the simple PO_4^{3-}. Chelate-type coordination of the oligophosphates was also proved by ^{31}P NMR measurements [41,49,50]. In the case of triphosphate, separate resonances of the free and complexed phosphates for both the terminal and the central phosphorus atom could be observed. [In most Al(III)–ligand systems, ligand-exchange reactions are slow on the NMR time-scale.] In the case of an excess of ligand and at pH ≈ 5, the occurrence of a new signal downfield to the previous ones [those of the free ligand and the 1:1 complex(es)] indicates rather unanbiguously the formation of the bis-complex.

Cyclotriphosphate and cyclotetraphosphate have been shown to be capable of binding Al(III) only through one phosphate group, via the formation of four-membered chelate rings with the PO_4 tetrahedron [51–53]. Hence their binding strength is more or less comparable to that of the monophosphate [log $K_{Al(P_3O_9)} \approx 3.1$, log $K_{Al(P_4O_{12})} \approx 4.5$] [52]. Owing to steric reasons, Al(III) can bind to two adjacent phosphate groups of the larger cyclohexaphosphate, which will result in a much higher binding strength [52].

5.3. *Al(III)–nucleotides*

Nucleotides contain three different metal binding sites: phosphate groups in the mono-, di- or triphosphate moieties, alcoholic hydroxyl(s) at the ribose or deoxyribose unit and carbonyl O and/or ring N donors in the nucleic base functions. The phosphate binding site can be weakly basic or non-basic, as in the nucleic acids, DNA and RNA, or it can be basic, as the terminal phosphate in nucleoside

phosphates and in many other biophosphates. Some stability constants recently determined for Al(III) binding to adenosine nucleotides [54] are given in Table 4.

The basic terminal phosphate group is the primary binding site for Al(III) [43,50,54–57]. No significant interaction with the other two binding sites has so far been detected by any structural investigation method. The stability constant for the formation of the equimolar complex AlL^-, the predominant species below pH $\approx$4.5, is greater than that for most metal ions, including Cu(III). In addition, Al(III) forms strong bis-complexes with a second ligand molecule [54,57]. Although the free nucleotides are minimally stacked in dilute solutions at millimolar concentrations, the charge neutralization provided by binding of Al(III) should promote base stacking, which may promote the formation of bis-complexes. As can be seen in the species distribution for the Al(III)–ATP system presented in Fig. 2, the mixed hydroxo mono complex $AlL(OH)^-$ predominates in the physiological pH range 7.0–7.4, besides the bis-complexes AlL^{5-} and $AlL_2(OH)^{6-}$, which however overlaps the concentration of the mono complex at higher ligand excess (see also fig. 1 in the paper by Nelson, p. 98 of this issue).

In the adenosine-5′-phosphate series, log K_{AlL} shows the sequence AMP< ADP$\leqslant$ATP, suggesting Al(III) chelation to the two terminal phosphate groups in ADP and ATP and coordination to the single phosphate in AMP. ^1H, ^{13}C, ^{31}P and ^{27}Al NMR measurements established the formation of two main species in slow exchange with the free ligand [55,58]. In the pH range 3.8–7.3, and at a 1:1 Al/ATP ratio, a dimeric (2:2) species was detected, while another species, a bis-complex, was formed in the pH range 4.2–8.1 in the case of an excess of the ligand. ^{31}P data revealed that Al(III) binding occurs at P_β and P_γ in the phosphate chain, resulting in complexes containing octahedral Al(III) coordinated to six O atoms, as suggested by ^{27}Al NMR measurements [55]. On the basis of ^1H NMR spectroscopic results, it was proposed that Al(III) binds to N(7) of the adenine base [50]. As discussed later [59,60], the observed upfield H(8) shift is due to nucleic base deprotonation at N(1) or base stacking rather than to Al(III)–N(7) coordination, which should result in a downfield shift. The contradiction between potentiometric

Table 4
Proton (log K) and aluminium (log β) stability constants of adenosine 5′-phosphates (AMP, ADP and ATP)[a]

Parameter	AMP	ADP	ATP
Log K_{HL}	6.04(1)	6.19(1)	6.31(1)
Log K_{H_2L}	3.74(2)	3.79(2)	3.89(2)
AlLH	–	10.98(4)	11.30(4)
AlL	6.17(1)	7.82(3)	7.92(4)
$AlLH_{-1}$	2.02(9)	2.94(8)	2.46(7)
AlL_2	10.35(11)	12.16(4)	12.47(4)
AlL_2H_{-1}	[b]	5.01(7)	4.84(5)

[a] Ref. [54], at I=0.2 M KCl and t=25 °C.
[b] Precipitation.

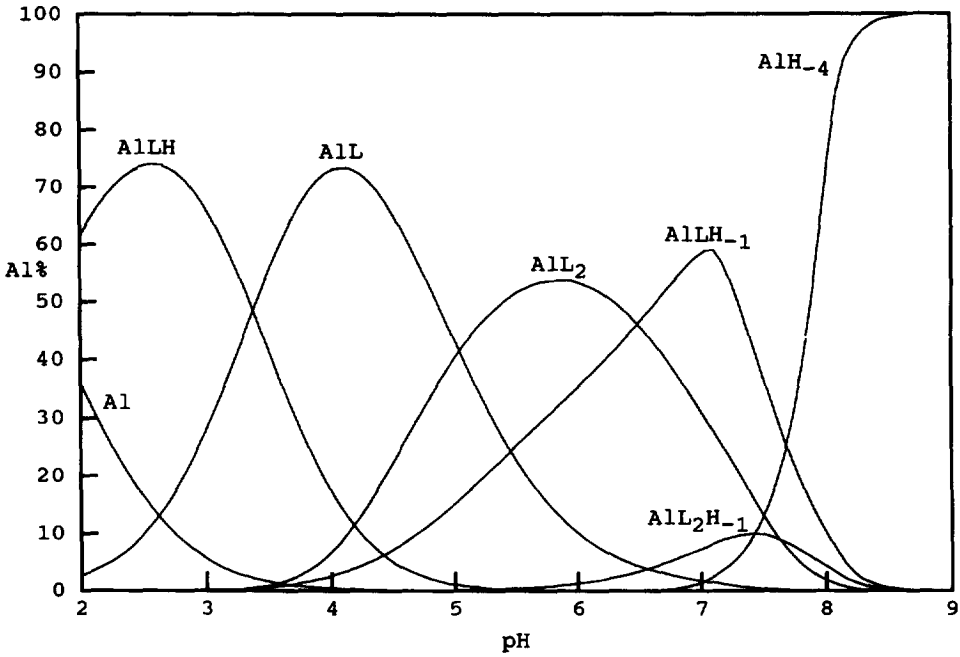

Fig. 2. Species distribution curves for 1 mM total Al(III) and 2 mM total ATP using the constants in Table 4. L refers to tetraanionic ATP^{4-}.

and NMR results concerning the monomeric or dimeric structure of the 1 : 1 complex appears to be only an apparent one, as NMR measurements were carried out on solutions one order of magnitude stronger, in the 10 mM Al(III) range (which would certainly favour dimer formation), while pH-metric measurements are not sufficiently sensitive to detect processes having no direct pH effect, such as the dimerization reaction $2AlL^- \rightarrow Al_2L_2^{2-}$, and thus cannot differentiate between AlL^- and $Al_2L_2^{2-}$. The formation of $Al_2L_2^{2-}$ via a stacking interaction between the adenine rings, however, is a very reasonable assumption. Most recent ^1H and ^{31}P NMR evidence for the formation of stacked 1:1 and 1:2 complexes in the Al(III)–ADP and Al(III)–ATP systems are discussed by Nelson elsewhere in this issue.

Al(III) binds to all nucleoside phosphates predominantly through the phosphate groups. The only basic phosphate group ($pK_a \geqslant 6$) is the terminal one. Since the nucleoside triphosphates exhibit a similar phosphate basicity to that of the nucleoside diphosphates and nucleoside monophosphates, the equilibrium constants in Table 4 can also be applied to other nucleoside phosphates. Great similarity in the Al(III) binding ability of various adenine and guanine nucleotides was observed by ^{27}Al NMR spectral measurements [49].

In contrast with nucleotides, nucleic acids contain only weakly acidic phosphates ($pK_a < 2$), whose metal binding ability is weak. The interaction of Al(III) with DNA was found to be so weak that a quantitative study was limited to pH < 5.5 because of metal ion hydrolysis and precipitation [61]. A stability constant log $K = 5.6$ was

suggested as the upper limit to characterize Al(III) binding to DNA under intracellular conditions. In early work [62,63], several mono- and dinuclear Al(III) complexes were detected with DNA at micromolar Al(III) concentrations using thermal denaturation and circular dichroism methods. The detailed structures of the Al(III)–DNA complexes remain to be specified. However, with very weakly basic phosphates, DNA serves merely as a polyelectrolyte interacting with Al(III) weakly and non-specifically. Log $K = 5.6$ is more than 3 log units less than the conditional stability constants for nucleotides containing basic phosphate binding sites [13]. Therefore, it is obvious that DNA cannot compete with ATP, other nucleotides or biophosphates for Al(III).

5.4. *Al(III)–2,3-diphosphoglycerate*

2,3-Diphosphoglycerate (DPG) is an important constituent of red cells. It is present at 2–4 mM concentration and thus, besides ATP, seems likely to be the predominant small molecule Al(III) binder in red cells. Potentiometric measurements [64] revealed that 1:1 and 1:2 complexes (see Fig. 3) are formed with Al(III), and complexation most likely occurs at the carboxylate-2-phosphate chelating site in both the mono- and bis-complexes.

In the successive deprotonation series $AlL_2H_2^{3-} \rightarrow AlL_2H^{4-} \rightarrow AlL_2^{5-}$, the two unchelated 3− charged phosphate groups deprotonate in a stepwise way. Fig. 3

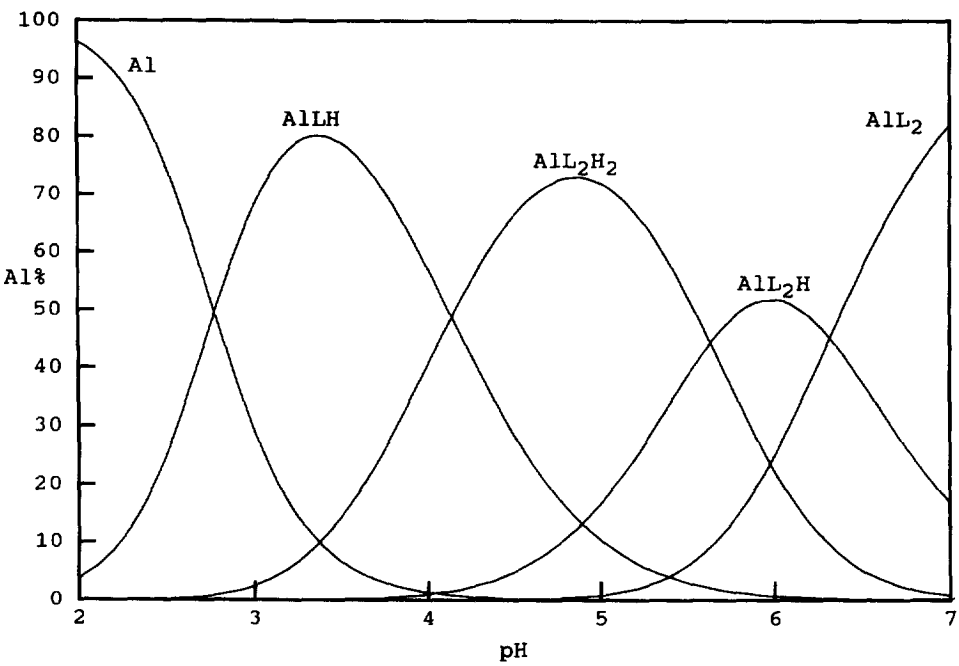

Fig. 3. Species distribution curves for 0.6 mM total Al(III) and 6 mM 2,3-diphosphoglycerate using the following log β values: HL, 7.14; H_2L, 13.10; H_3L, 16.29; AlLH, 13.12; AlL_2H_2, 24.42; AlL_2H, 18.78; AlL_2, 12.46.

shows that under physiological conditions a neutral solution of DPG chelates Al(III) in the fully deprotonated complex AlL_2^{5-}. The stability constant of this complex is log $K_{AlL2} = 12.5$, which is comparable to that of ATP (see Table 3), confirming the potential Al(III) binding ability of both ligands in blood cells. Speciation model calculations demonstrate that free "Al^{3+}" concentrations allowed by the most likely Al(III) binders ATP and 2,3-DPG in the cells and citrate and transferrin in the blood plasma are about the same $[Al^{3+}] = 10^{-14}$ M. Therefore, at equilibrium there should be comparable concentrations of Al(III) on both sides of the red cell membranes.

5.5. *Al(III)–phosphorylated proteins*

Phosphorylated proteins are important constituents of different body tissues, and are particularly important in brain cells, such as in the nuclear chromation region. Phosphorylation and dephosphorylation reactions normally accompany cellular processes. The basic phosphate groups of any phosphorylated protein provide the necessary basicity and, in conjunction with juxtaposed carboxylate or other phosphate groups, become strong Al(III) binding sites [38]. Although there are no quantitative stability data characterizing the Al(III) binding strength of these biomolecules, we can estimate a value of log $K \approx 6$ for the interaction of a single phosphate group with an Al(III) centre. If other binding donors, such as a carboxylate or another phosphate, are also present in the proximity of this binding site, the stability constant can reach, as an upper limit, those of the 2,3-diphosphoglycerate or diphosphate complexes (see Fig. 3 and Table 4). In vitro circular dichroism studies have revealed that Al(III) is able to induce significant conformational changes in phosphorylated or overphosphorylated (9–100 mol of phosphate per mole of protein) neurofilament proteins, yielding a high content of β-pleated structure [65]. This unambiguously suggests a direct interaction between Al(III) and the strongest binding site of the phosphorylated protein, the phosphate group [66]. Earlier investigations proved that ^{31}P NMR can be a very efficient and sensitive tool for the detection of such metal ion-induced changes [67]. In a recent enzymatic study phosvitin, the major phosphoglycoprotein of egg yolk, was used as a model for studying the interaction of Al(III) with multiphosphorylated proteins [68]. The results demonstrated that the effect of Al(III) on phosvitin showed significant similarities with the action of this metal ion on neurofilament proteins.

5.6. *Al(III)–phospholipids*

Phospholipids are the most abundant constituents of all biological membranes. They are derived from either glycerol or sphingosine, both polyalcohols. Phosphoglycerides consist of a glycerol backbone, two fatty acid chains and a phosphorylated alcohol. Thus, in contrast to phosphorylated proteins, they contain only weakly acidic 1− charged phosphate groups; their metal binding ability is much weaker. Biophysical studies [69–71] have shown that Al(III) at micromolar concentrations promotes aggregation, fusion and membrane rigidification, which is not limited to membranes composed exlusively of negatively charged phospholipids. Similar

changes could be induced in phosphatidylserine-, phosphatidylethanolamine- and phosphatidylcholine-containing lipid vesicles. This strongly suggests a role of the direct interaction of Al(III) with the phosphate binding site. On the basis of a comparative study on the membrane aggregation and fusion promoting effectiveness of the dipositive Ca^{2+} and the tripositive Al^{3+} it was estimated that 20–50 μM "Al^{3+}" with an association constant $\log K \approx 4$ could displace nearly all divalent cations from the membrane under physiological conditions [69,72]. In a recent in vitro study, the direct interaction of various Al(III) complexes with dipalmitoylphosphatidylcholine was clearly detected by ^{31}P NMR spectroscopy in aprotic solvent, which mimic much better the hydrophobic membrane environment [73].

5.7. *Al(III)–myo-inositol phosphates (IP)*

D-*myo*-Inositol 1,4,5-triphosphate acts in a wide variety of cells by triggering the release of sequestered Ca(II) from the endoplasmic reticulum after stimulation of a specific intracellular receptor [74]. Another IP, D-*myo*-inositol 1,2,6-triphosphate, displays promising pharmacological properties resulting from its extracellular action, which has still to be clarified [75]. By considering their chemical structure, which consists of three basic phosphate groups bound around the inositol ring, they appear to bind most of the metallic cations present in biological fluids. A comparative speciation study revealed that both inositol triphosphate isomers form similar complexes (see Table 5) and under physiological conditions they bind Al(III) more strongly than does ATP [75].

Phytic acid (*myo*-inisitol hexaphosphate) readily forms insoluble complexes with essential metal ions, thereby affecting their bioavailability. It was found that Al(III) forms both soluble and insoluble phytate complexes, presumably with 1:1 and 4:1 Al–phytate stoichiometries, respectively [76]. The reaction heats were endothermic, and the interaction of one Al(III) with one phosphate group could be characterized by $-29\ kJ.mol^{-1}$ enthalpy changes in both complexes.

Table 5
Proton (log K) and aluminium (log β) stability constants of *myo*-inositol triphosphates[a]

Parameter	l(1,4,5)P[b]	l(1,2,6)P[b]
Log K_{HL}	8.74	9.48
Log K_{H_2L}	7.02	7.22
Log K_{H_3L}	5.80	5.70
Log K_{H_4L}	2.61	2.40
AlLH	18.98(6)	19.18(3)
AlL	13.37(7)	13.72(3)
AlL_2	–	19.72(7)
$AlLH_{-1}$	5.84(8)	6.10(4)
$AlLH_{-2}$	1.82(10)	–

[a] Ref. [75], at $I=0.1$ M tetra-*n*-butylammonium bromide, $t=25\,°C$.
[b] l(1,4,5)P, DL-*myo*-inositol 1,4,5-triphosphate; l(1,2,6)P, D-*myo*-inositol 1,2,6-triphosphate.

5.8. Al(III)–phosphate–ligand B ternary systems

As real biological systems always contain many potential metal ion binder biomolecules, the formation of ternary complexes may have much greater importance than that of simple binary species. In spite of this obvious fact, little attention has been paid to Al(III) ternary systems including biophosphates and other ligands B. This may be explained by the relatively complicated nature of the Al(III) binary systems (as discussed above) and the technical and methodological problems connected with the slow formation reactions and strong hydrolytic tendency of Al(III).

The interaction of Al(III)–nucleotides with F^- served as a model to study whether AlF_4^-, a tetrahedral pseudo-phosphate, can bind to GDP [or other nucleoside diphosphates (NDP)] in G-protein systems. In a recent study, various ternary complexes (NDP)AlF_x ($x=1$–3) were identified by using ^{19}F and ^1H NMR techniques, but no (NDP)AlF_4 was found [77]. Ternary complexes were formed with a frequency predicted statistically on the basis of binary complex stabilities (for more details, see the papers by Martin and by Nelson in this issue).

The possibilities of ternary complex formation have been studied in the Al(III)–adenosine-5′-phosphate (AMP, ADP and ATP)–ligand B (oxalic acid, lactic acid, and malic acid) systems by pH-potentiometric and ^{31}P NMR methods [78]. The formation of ternary complexes AlLBH and AlLB was favoured in all systems in the acidic pH range. Under physiological conditions, however, Al(III) was bound mainly to the nucleotides; almost exclusively in the presence of the relatively weak bidentate Al(III) binders oxalic acid and lactic acid and about 30% in the presence of the much stronger tridentate coordinating molecule malic acid (see Fig. 4).

Al(III)–phosphate–citrate ternary complexes are assumed to be potential low molecular weight Al(III) binders in plasma [38]. Although there are indications of ternary complex formation in the Al(III)–PO_4^{3-}–citrate system at acidic pH, all our efforts to detect ternary complexes in the Al(III)–ATP–citrate and Al(III)–PO_4^{3-}–citrate systems at physiological pH have so far failed. The relatively unfavoured formation of Al(III) ternary complexes at neutral and weakly basic pH might be connected with changes in the geometry of the complexes from octahedral to tetrahedral when OH^- ions can also be involved in the coordination, or with the different geometries of the binary species. In contrast, ternary complex formation was detected by potentiometry in the Al(III)–triphosphate–amino acid systems [79]. However, the improper treatment of the binary systems may raise the question of the reliability of the observations. It may be concluded from the above-mentioned results that more reliable data on relevant model systems would be necessary to draw any general conclusion on the actual importance of ternary complex formation in Al(III) binding processes in biological fluids.

6. Biological implications

The bonding interaction of Al(III) with PBB is evidently a crucial point in aluminium biology. On considering the list of cellular and molecular implications in

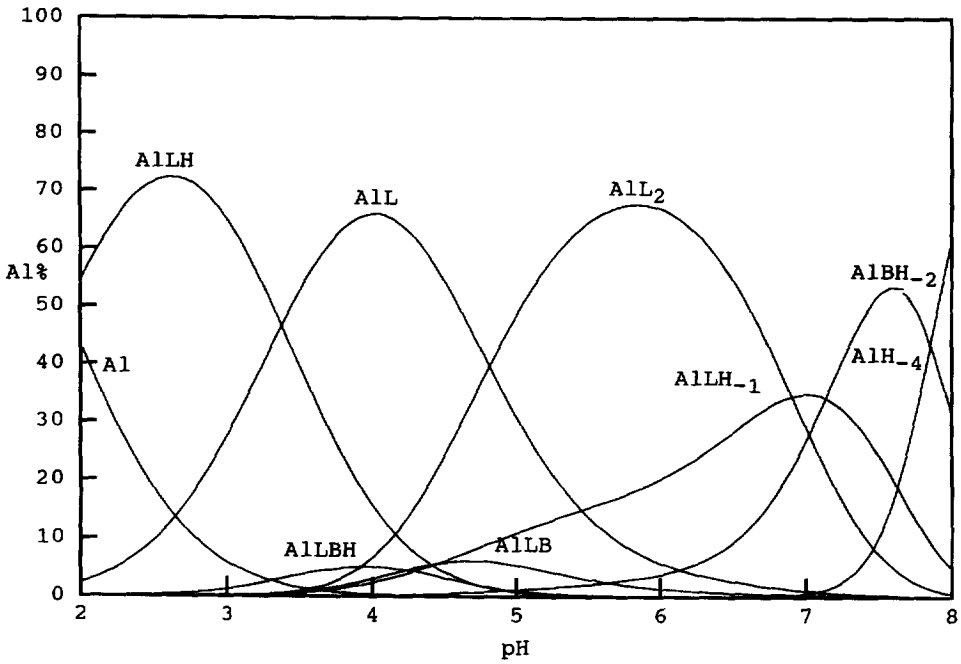

Fig. 4. Species distribution curves for 1 μM total Al(III), 1 mM ATP (L) and 1 mM malic acid (B) using log β values given in Table 4 and HB, 4.57; H_2B, 7.73, AlBH, 7.34, AlB, 3.8, $AlBH_{-1}$, 1.32; $AlBH_{-2}$, −3.74, AlB_2H_{-1}, 4.74; Al_2BH_{-3}, −3.84; AlLBH, 15.52; AlLB, 11.29.

this review and the series of phenomenological observations dealing with aluminium toxicology outlined in the other paper from our group in this issue (pp. 11–22), we speculate about possible rationales able to shed light on the connection between Al(III) and important human and experimental pathologies: Alzheimer's disease (AD), dialysis dementia (DD) and experimental encephalopathy (EE). In this connection, wherever Al(III) accumulates in a cell population, the process is expected to be irreversible and no relevant detoxification processes are known to date.

In AD, distrophic neurons (affected by neurofibrillary tangles [36]) and brain areas impaired by senile plaques [37] (aggregates of protein material not yet fully elucidated, affecting the brain mass in a "patchwork" fashion) are evident in the neurocortex and hyppocampus, in which these crucial features of AD develop very slowly in 5–10 years, both being associated with focal concentrations of Al(III) (see above). The significant overall elevation (2–3-fold) [5] may be the result of an impairment of the blood–brain barrier (BBB) for which event Al(III) is known to be active [15,16], but not necessarily the cause. This proposed lack of efficiency of BBB may well stem from genetic or environmental reasons. Excess Al(III) in the extraneuronal fluids may reach the more vulnerable neurons and act along four aggression lines: (i) destabilizing the plasmatic membrane, thus predisposing neuron disintegration; (ii) stimulating the hyperphosphorylation of the neurofilaments [35], thus making them more prone to a cross-linking effect by Al(III) itself [23,27];

(iii) undergoing selected coordination by hyperphosphorylated histones proteins [65], which will impair the regular neurofilaments synthesis and produce the typical pair-helical shaped ones [6]; and (iv) interfering with the regular neurofilaments turnover upon inhibiting the neuronal proteolytic activity [80]. The consequent slow neuronal death and disintegration would add to the well established abnormal formation of the mainly extracellular amyloid material of the senile plaques, in which the presence of polypeptide domains typical of membrane proteins has been proved [81].

In DD, the elevation of physiological aluminium levels is dramatic (10–20-fold) and relatively fast (2–4 years). Most remarkably, DD is a disease sporadic in nature, i.e. uraemic patients treated in similar ways and undergoing a similar brain aluminium loading do or do not develop DD [1]. In DD Al(III) is likely effectively to attack the BBB, thus making possible a relatively large flooding of Al(III) towards the various brain cells (neurons, astrocytes, glias). Under these conditions, the most rapid cellular event may be the aggression to neurons, whose lysosomes would become the target for the invading metal centre, with consequent cellular degeneration and general impairment of brain functions. As mentioned above, the hypothesis of Al(III)-induced neurons autolysis *in vivo* was documented in 1984 [22], but subsequent experiments in vitro [82] gave unclear results. Again, the sporadic nature of DD may be linked to the genetic predisposition of Al(III) uptake at the level of BBB.

In EE, animals are subjected to a relatively very rapid and massive exposure to Al(III). In several cases Al(III) was directly injected into the animal brain, more precisely into the brain ventricles [83]. As pointed out in the other paper from our group in this issue (pp. 11–22), the following cascade of chemo-clinical, histopathological and clinical observations have been described: (i) metal accumulation in nuclear chromatin (as in AD); (ii) neurofibrillary degeneration in hyppocampal neurons (as in AD), but without senile plaques; (iii) behavioural deficits. However, the ultrastructure of the degenerated neurofilaments is substantially different from that observed in AD. It appears that the toxic aspects of drastic aluminium intoxication in sensitive animals (mainly rabbits) [6] are more similar to (or less different than) those of AD rather than to those of DD. Two facts should be considered however: (i) sensitive animals and humans may not be identically sensitive, in terms of molecular biology, to Al(III) aggression owing to philogenetic reasons [84], so that features such as senile plaques and the peculiar neurofilament degeneration typical of AD might not be *in principle* generated in animals, and (ii) Al(III) accumulation and toxic effects in the glias of intoxicated rabbit brain have not yet been sought.

Acknowledgements

This work was sponsored by the US Hungarian Science and Technology Joint Fund in cooperation with the Department of Health and Human Services, USA, and Ministry of Social Welfare, Hungary, under Project No. 182/92b and by the National Science Research Fund, Hungary, under Project No. OTKA/T7458.

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