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Speciation and structural aspects of interactions of Al(III) with small biomolecules

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Contents

Abstract

Complexes formed with low molecular mass biomolecules are the 'dynamic or mobile units' of Al(III), which may be involved in the absorption and transport processes of this toxic element in organisms. This paper reviews the interactions of Al(III), from speciation and structural aspects, with biologically relevant endogenous and exogenous small biomolecules such as inorganic ligands (hydroxide, fluoride, (oligo)phosphates and silicic acid), amino acids, phosphorylated amino acids, oligopeptides, biophosphates including nucleotides, phosphonates, hydroxamates, and aromatic and aliphatic hydroxycarboxylates. The importance of time in biospeciation is demonstrated on the examples of binary and ternary systems involving Al(III) and citric acid. Examples are also given for the implications of the speciation of Al(III) with such small biomolecules in biology.

Keywords: Al(III) complexes; Aluminium biospeciation; Low molecular mass biomolecules

1. Introduction

It is clear that Al is a toxic element in plants, animals and humans [1]. In order to understand the effects of Al in the environment and organisms, we need to know not only the overall amount present, but also the multitude of forms in which this element may exist. The speciation or identification of chemical species of Al(III) is essential [2]. It is now well understood that the toxicity of Al(III) in aquatic and terrestrial systems does not correlate well with the total Al(III) concentration, but is rather a function of the concentration of the biologically active form [3]. In terms of acute toxicity, the inorganic forms are believed to be more toxic.

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However, organically bound species may be capable of crossing biological membranes and contributing to chronic bioaccumulation. Additionally, partly independently of the chemical form in which it enters the given organism, it will interact with many endogenous and exogenous potential binders of the various biofluids and tissues. Accordingly, speciation studies are necessary to determine the chemical forms in which Al(III) is present in biological systems [4,5].

Fig. 1 depicts the various routes through which Al can enter the human organism from the environment, the diet or medication. Al (10–30 mg) is estimated to be ingested each day, but only a very small proportion of this Al (5–10 μg) is absorbed. In many countries, aluminium sulfate is used as a coagulant in water treatment. Acid rain increases the Al load from the environment considerably. Al(III) compounds, e.g. alums, are used as additives in different food-producing processes, for instance as baking powder in some countries. Some plants, e.g. tea, can accumulate an enormous amount of Al: the Al content of old tea leaves can reach even 3%. A significant amount of Al(III) may be liberated from Al vessels used in the kitchen when acidic meals are cooked in them. Some antacids and buffered medicines also contain Al and this may be the greatest potential source of aluminium intake. Al can accumulate to a dangerous extent in patients with an impaired renal function, who are treated with orally taken Al(III)-containing phosphate binders. Some forms of medical treatment, e.g. long-term haemodialysis, can also elevate the Al level in humans if the water used has a high Al content. Dialysis centres now test water for Al(III) content. Under normal conditions, i.e. with a normal daily load and a healthy organism, the Al intake is well tolerated (although the physiological effects of a long-term low aluminium load, e.g. from the drinking water, are still not clear [6]) in consequence

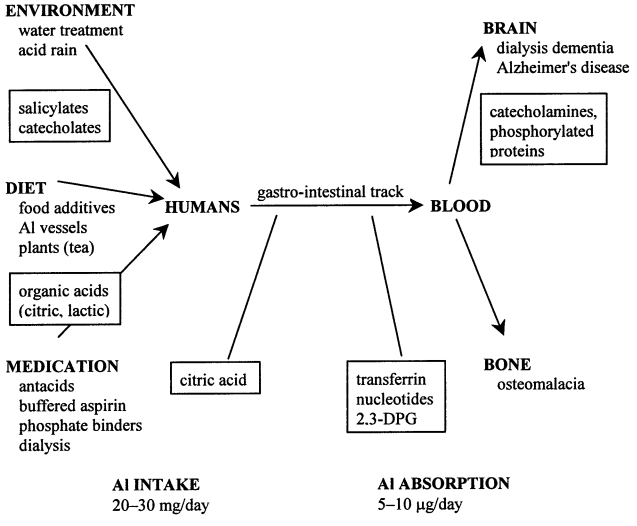


Fig. 1. Various routes of Al(III) entering the body (from Ref. [5]).

of the formation of poorly-soluble phosphates and hydroxides in the gastro-intestinal tract. These compounds are easily excreted and are therefore not harmful. Most of what is absorbed is eliminated in the urine (probably in the form of a citrate complex). In the event of an abnormally high Al load and/or with an impaired renal function, however, an excess of Al(III) can be absorbed and transported to various target organs, where it may accumulate and exert harmful effects, e.g. osteomalacia in the bones, microcytic anaemia in the red blood cells or neurodegenerative diseases in the brain.

Al(III) is a typical hard metal ion, and the most likely binding sites of $[Al(H_2O)_6]^{3+}$ in biosystems are therefore O donors, and especially negatively-charged O donors. Carboxylate, phenolate, catecholate and phosphate are the strongest Al(III) binders. Biomolecules containing such functions may be involved in the uptake and transport processes, and also in the biological and physiological actions of Al(III) in living systems. These small biomolecules (some of them are included in Fig. 1) are either of biological importance or can mimic larger biomolecules. For example, salicylate and catecholate derivatives can serve as good model compounds via which to study the metal-ion binding properties of the large soil organic materials, humic and fulvic acids. Small organic acids, e.g. as citric acid, are important low molecular mass binders in biological fluids. Transferrin plays an essential role in Al(III) transport in the plasma. 2,3-Diphosphoglycerate is important in the binding and accumulation of Al(III) in the red blood cells, resulting in a special kind of anaemia. Other phosphates, such as nucleoside phosphates, e.g. ATP, are widely distributed in living systems, and numerous of the reactions, which take place in living organisms, involve ATP. Catecholamines occur in fairly high concentration in the brain and may have an important role in binding Al(III). Phosphorylated peptides and proteins, which are frequently observed among neuron degradation products in various neurological disorders, may play a role in the accumulation of Al(III).

In addition to the stability of metal ion complexes, an important and often overlooked feature is the rate of ligand exchange out of and into the metal ion coordination sphere. Kinetically, ligand-exchange reactions for the Al(III) ion and its complexes may be characterised as sluggish. Ligand-exchange reactions will definitely occur in vivo. However, the rate of ligand exchange may be comparable to the rates of transport and other biological processes, and thus, biological fluids may not attain their true thermodynamic species distribution. This is particularly true for the formation of polynuclear complexes. The reactions of oligonuclear and/or mixed hydroxo complexes of Al(III) can be extremely slow, resulting in a non-equilibrium state of Al(III)–lig-

and systems in biology. Accordingly, the time dependence of the speciation of Al(III) complexes, which may provide a non-equilibrium species characterisation of Al(III) in biological fluids and tissues, may be more relevant to biological systems.

This chapter reviews the speciation and structural aspects of the interactions of Al(III) with small biomolecules which might be relevant to the occurrence of Al(III) in biology. Since these small species are the 'dynamic or mobile units' through which Al(III) can be translocated in vivo, it is of particular importance to know which of them predominate under specific circumstances. Accordingly, the complexes of relevant biomolecules such as inorganic ligands, amino acids, peptides, biophosphates, and aliphatic and aromatic hydroxycarboxylates are reviewed. The importance of the role of time in biospeciation will be demonstrated in connection with binary and ternary systems involving Al(III) and citric acid. As an exhaustive review on this topic was published by Kiss and Farkas [7] in 1996, we focus in the present work on the literature reported since 1995.

2. Complexes with inorganic ligands

As a highly charged small cation, Al^{3+} is readily hydrolysed in aqueous solution in the absence of competing ligands. In solutions more acidic than pH $\sim$ 5, Al(III) remains unhydrolysed, the main mononuclear species being $[Al(H_2O)_6]^{3+}$, usually abbreviated as Al^{3+}. As the pH increases, mononuclear species such as $[Al(OH)]^{2+}$, $[Al(OH)_2]^{+}$ and soluble $Al(OH)_3$ are formed as well as polynuclear species, depending on the time and the total concentration of Al(III) (a high Al(III) concentration favours oligomerisation reactions, which are usually rather slow processes). The available evidence [7–9] suggests that the most important polynuclear species are $[Al_2(OH)_2]^{4+}$, $[Al_3(OH)_4]^{5+}$ and $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$. However, there are indications for the existence of other oligonuclear complexes [10–12], the formation of which is strongly concentration dependent. Neutral solutions give a precipitate $Al(OH)_3$ that redissolves in consequence of the formation of $[Al(OH)_4]^{-}$, the primary soluble Al(III) species at pH > 7 at a micromolar level of total Al(III). If there is no other ligand present that is capable of holding Al(III) in solution, the solubility equilibria for $Al(OH)_3$ [1] and the soluble hydroxo complexes of Al(III) must be taken into account in order to describe the solution state of Al(III) in a given sample.

The fluoride ion is a unique ligand in that it forms more stable complexes with Al(III) than with Fe(III). The speciation and stability of the Al(III)–fluoro complexes have been critically reviewed [13]. It has been clarified that $[AlF_4]^{-}$, proposed to be a tetrahedral phosphate analogue, is actually a hexacoordinated octahedral species, with the stoichiometry $[AlF_4(H_2O)_2]^{-}$. Tóth and coworkers [14] recently investigated the equilibria and dynamics of the Al(III)–F^{-} system by means of ^{19}F-NMR spectroscopy. While the pentacoordinated complex $[AlF_5]^{2-}$ could be clearly detected, the formation of hexacoordinated species could not be proved. Since OH^{-} and F^{-} are isoelectronic and isochoric and display many parallelisms in their chemistry, both in solution and in the solid state, it is surprising that their interaction with $[Al(H_2O)_6]^{3+}$ should be so dissimilar. As noted by Tuck [15], this is a reflection of the very finely balanced strengths of interaction of Al^{3+} with the three hard donor ligands H_2O, OH^{-} and F^{-}.

It is to phosphate groups that Al(III) is frequently and significantly complexed in biological systems. At neutral pH, Al^{3+} readily forms a poorly soluble compound with inorganic phosphate. Though often formulated as $AlPO_4$, this species appears to be a mixed phosphate–hydroxo complex with variable proportions of phosphate and hydroxide [16]. Such species provide one of the major modes of elimination of ingested Al(III). Precipitation of these complexes limits the concentration of soluble Al(III)–phosphate–hydroxo species at neutral pH to $\sim$20 μM [17]. The solution chemistry of this system can be studied directly by pH-potentiometry only below pH 4. In this pH range, the formation of mononuclear complexes $[Al(H_2PO_4)]^{2+}$, $[Al(HPO_4)]^{+}$ and $[Al(PO_4)]$ [18] and various oligonuclear species such as $[Al_2(PO_4)]^{3+}$, $[Al_2(PO_4)(OH)_2]^{+}$ [19], $[Al_3(HPO_4)_3]$, $[Al_3(HPO_4)_2(PO_4)]$ and $[Al_3(PO_4)_3]$ [20] and $Al_3(OH)_q(H_3PO_4)_r^{9-q}$ with $(q, r) = (6, 1)$, $(5, 2)$, $(6, 3)$, $(8, 3)$, $(6, 4)$ and $(8, 5)$ [21] has been reported.

LFER (linear free energy relationship: a linear relationship can be expected between the proton-binding ability of a given donor group, characterised by the appropriate pK value, and its ability to coordinate to metal ions) has been used to estimate binding constants for Al(III)–phosphate complexes [22,23] assumed to be formed under physiological conditions. Harris concluded that the hydrolysis of $[Al(PO_4)]$ is so strong that $[Al(PO_4)(OH)]^{-}$ will be the predominant monomeric Al(III)–phosphate complex at neutral pH. However, attempts to estimate the binding constants for $[Al(PO_4)]$ and $[Al(PO_4)(OH)]^{-}$ from LFER are complicated by two questions: is phosphate monodentate or bidentate in solution and should these species be formulated as shown or as $[Al(HPO_4)(OH)]$ and $[Al(HPO_4)(OH)_2]^{-}$ [17]? At present, there are no generally accepted Al(III)–phosphate binding constants that describe complexation under physiological conditions. Our log β recommendations are as follows: $[Al(HPO_4)]^{+}$: 17.6, $[Al(HPO_4)(OH)]$: 13.5, $[Al(PO_4)(OH)]^{-}$: 7.2 [23]. When the corresponding p$K(HPO_4^{-})$ value is taken into account, the equilibrium constant characteristic of the

reaction $Al^{3+} + HPO_4^- \rightleftharpoons (AlHPO_4)^{2+}$, representing the interaction of Al(III) and inorganic phosphate, is $\log K = \log \beta(AlHPO_4)^+ - pK(HPO_4^-) = 17.6 - 11.5 = 6.1$, i.e. slightly stronger than that characteristic of the Al(III)–amino acid interaction. Although an unambiguous speciation description of this simple system is still awaited, it is very likely that, besides these mononuclear complexes, dinuclear species, such as $[Al_2(PO_4)]^{3+}$, $[Al_2(PO_4)(OH)]^{2+}$ and $[Al_2(PO_4)(OH)_3]$ or others [24] may also be present in stronger solution. The numerous ^{31}P- and ^{27}Al-NMR resonances observed in the NMR spectra seem to support this assumption [23,25–27].

A relatively recent ^{27}Al-NMR study on the complexation of Al(III) with phosphinate ($H_2PO_2^-$) and phosphite (HPO_3^{2-}) ions between pH 1 and 3 must be mentioned [28]. The formation of 1:1, 1:2, 1:3 and 2:2 complexes was detected by means of ^{27}Al-NMR experiments; the stability constants of these complexes are 102, 19.2, 18.2 and 12.9, respectively. For the Al(III)–phosphite system, the 1:1, 1:2 and 2:2 complexes have stability constants of 102, 17 and 163, respectively. The geometry of the dimeric complex is discussed on the basis of geometry optimisation calculations: the structure in which the Al(III) ions are linked by two phosphinates, with each O atom bound to one Al(III), seems to be most probable. These results coincide with the earlier findings of Feng and Waki [29], obtained by ^{31}P-NMR spectroscopy.

Diphosphate (DP), triphosphate (TP) and other oligophosphates can form stable six-membered mono- and bis-chelates with Al(III) via the coordination of adjacent phosphate groups [23]. They bind Al(III) about four orders of magnitude more strongly than can monophosphate (see Table 1). It is noteworthy that, in contrast with PO_4^{3-}, oligophosphates readily form bis-chelated complexes, although the spatial requirements of the ligand molecules and also the electrostatic repulsion due to the coordination of a highly charged second ligand molecule are larger than in the case of the simple PO_4^{3-}. Several signals are detected in the ^{31}P-NMR spectra of Al(III)–DP solutions: one for the free ligand, the chemical shift of which depends on the pH (from -10.8 ppm with respect to 85% H_3PO_4 in D_2O at pH 2.1 to -7.4 ppm at pH 7.5), one at -10.9 ppm assigned to $[AlLH_2]^+$ (monodentate), one at -11.7 ppm for $[AlL_2H]^{4-}$, one at -13.2 ppm for [AlLH] (chelate), one at -8.5 ppm assigned to $[AlLH_{-1}]^{2-}$ and finally one at -9.1 ppm attributed to $[AlL_2]^{5-}$. For the Al(III)–TP system, the ^{31}P-NMR spectra are more complex since the ligand itself gives rise to two peaks (a doublet for the α- and γ-P, and a triplet for the central P). The coordination of Al(III) either to DP or TP broadens the ^{31}P-NMR signals and the resonances are shifted upfield, with the exception of that of the β-P atom in TP, which is moved downfield.

Table 1
Acidity constants ($-\log K$) and Al(III) stability constants ($\log \beta$)

	MP	DP	TP
$-\log K(H_3L)$	1.86	1.67	1.70
$-\log K(H_2L)$	6.63	5.87	5.34
$-\log K(HL)$	11.48	8.23	7.76
$\log \beta(AlLH_2)$	–	18.69	18.07
$\log \beta(AlLH)$	17.6	17.03	16.65
$\log \beta(AlL)$	13.5	13.74	13.15
$\log \beta(AlLH_{-1})$	7.2	7.41	6.53
$\log \beta(AlL_2H)$	–	25.64	24.43
$\log \beta(AlL_2)$	–	19.77	19.14
$\log \beta(Al_2L)$	16.65	–	–
$\log \beta(Al_2LH_{-1})$	14.21	–	–
$\log \beta(Al_2LH_{-3})$	7.42	–	–

Complexes of monophosphate (MP), diphosphate (DP) and triphosphate (TP) ions at 25 °C and $I = 0.2$ M (KCl), data are from [23]. The β_{pqr} constants refer to the following reaction: $pAl + qL + rH \Leftrightarrow Al_pL_qH_r$.

It has been demonstrated that silicic acid significantly reduces the biological availability and toxicity of Al(III) [30,31]. This seems to involve the formation of hydroxyaluminosilicates (HAS) via competitive condensation: $Si(OH)_4$ competes with $Al(OH)_3$ to condense with preformed hydroxyaluminium templates. The formation and stability against the aggregation of HAS depend critically on the silicic acid concentration, the Al(III) concentration and the pH [32,33]. HAS may be formed in the blood serum too [34] if elevated Al(III) and silicic acid levels are present. The aluminium detected in senile plaques and neurofibrillary tangles in the brains of Alzheimer's disease (AD) patients is believed to involve aluminium silicates [35]. However, it has also been suggested that silicic acid might be able to solubilise Al(III)-induced plaques and tangles with a β-pleated sheet conformation through the formation of random coil soluble complexes [36]. To check this idea, the interaction of Al(III) with silicic acid was studied in the presence and absence of phosvitin (modelling phosphorylated brain proteins) [37]. It was found that silicic acid does not remove Al(III) from phosvitin and that phosvitin does not remove Al(III) from a mature precipitate of Al(III) silicate, although it does remove Al(III) if added to Al(III) freshly associated with silicic acid. This relationship between Al(III), silicic acid and phosvitin is explained by the kinetics of aging of Al(III)–silicic acid precipitates.

3. Complexes with amino acids, phosphorylated amino acids and oligopeptides

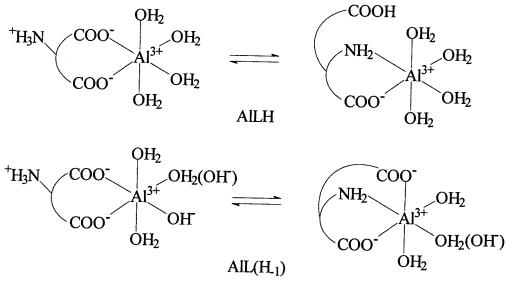
The α-carboxylate group of amino acids is weakly basic ($pK \sim 2.2$), which suggests a rather weak Al(III)-binding ability. The usual sample composition in the

pH-potentiometric titration method (a metal ion concentration at the millimolar level, with a metal ion to ligand ratio from 1:1 to 1:5) is not suitable for the unambiguous detection of complex formation [38]. It has been found that the strength of complexation to Al(III) decreases strongly in the sequence dicarboxylic acid ≫ hydroxycarboxylic acid > carboxylic acid ≫ amino acid. The weakening effect of amino substitution can be explained in terms of the electrostatic repulsive effect of the $-NH_3^+$ group. Use of a LFER approach led to a stability constant of $\log K \sim 5.8–5.9$ being estimated for the interaction between Al(III) and glycine, the simplest α-amino acid. According to this estimate, the coordination of Al(III) should become distinguishable from its hydrolysis at amino acid concentrations greater than 25 mM. In precise pH-metric studies at such high ligand concentrations and at high excesses of ligand (up to 1:40), simple α-amino acids such as glycine, alanine, serine, threonine, asparagine and glutamine were found unambiguously to influence the speciation of $[Al(H_2O)_6]^{3+}$ [39]. Formation of the mononuclear species $[Al(LH)(OH)]^{2+}$ (LH is an amino acid protonated at the amino end) and $[AlL(OH)]^+$ and a carboxylate and dihydroxo-bridged dinuclear complex $[Al_2LH(OH)_2]^{4+}$ may be assumed. The stability constants obtained from direct pH-metric measurements for the interactions of Al(III) with simple bidentate amino acids are in the range $\log K = 5.5–5.9$, which is in good agreement with the value derived from LFER calculations (vide supra). ^{27}Al-, ^{1}H- and ^{13}C-NMR studies [39,40] in the weakly acidic pH range, indicated that in the complexes $[Al(LH)(OH)]^{2+}$ and $[Al_2(LH)(OH)_2]^{4+}$ the simple amino acids bind in a monodentate way through the carboxylate group [41] and the amino group is in the protonated form. At pH ≥ 4.5, the solution becomes opalescent and $Al(OH)_3$ slowly precipitates. With a less basic amino group ($pK(NH^+) \sim 5.2$), complexation is conveniently observable with α-picolinate, which yields the mono- and bis-complexes $[AlL]^{2+}$, $[AlL_2]^+$ and $[AlL_2(OH)]$ [40,42]. The presence of an extra OH group in ring position 3 (3-OH-picolinic acid) makes the ligand an even stronger binder of Al(III) [43]; the ligand prefers picolinate-type (N, COO^-) coordination in the weakly acidic pH range, while through a rearrangement of the ligand, the salicylate-type (COO^-, O^-) binding mode occurs in basic solution [44]. Interestingly, quinolic acid (3-carboxypicolinic acid) forms only picolinate-type (N, COO^-) complexes, and formation of the phthalate-type (COO^-, COO^-) coordination remains subordinate, presumably because of the much lower stability of a seven-membered chelate ring [45].

The tridentate aspartic acid [Asp, $(COOH)CH_2$-$CH(NH_2)COOH$], containing two COO^- and one central NH_2 binding donors, is a significantly stronger Al(III) binder; the stability of the 1:1 complex is about two orders of magnitude higher than that of any simple amino acid, indicating the involvement of both carboxylates in metal binding [39]. It is also interesting that no such strong complexation has been detected with either succininc acid [$(COOH)CH_2CH_2COOH$] or *N*-acetylaspartic acid (both lack the central amino binding site), which would indicate involvement of the $-NH_2$ group in the binding mode of Al(III)–Asp complexes. Stepwise complexation, with subsequent formation of a monodentate carboxylate–coordinated complex and, through deprotonation, that of a chelate complex of Asp was strongly suggested by a kinetic investigation of the Al(III)–Asp system [46]. The possible binding isomers of the species $[Al(LH)]^{2+}$ and $[AlL]^+$ are shown in Scheme 1. The further proton liberation, resulting in the species [AlL(OH)] and $[AlL(OH)_2]^-$, can be ascribed to water molecules in the coordination sphere of the metal. The strong interaction between Al(III) and Asp can be detected by means of ^{27}Al-NMR: a relatively sharp signal at ~10 ppm (as compared to $[Al(H_2O)_6]^{3+}$) suggests octahedral Al(III) in a fairly symmetrical chemical environment. This spectral behaviour is reminiscent of that of the trinuclear Al(III)–citrate complex [47,48].

The tridentate coordinating ability of glutamic acid (Glu, $[(COOH)CH_2CH_2CH(NH_2)COOH]$) is much weaker, due to the lower stability of the seven-membered chelate ring, formed with participation of the terminal carboxylate group. Thus, Glu rather acts as a bidentate ligand, showing similarity with simple amino acids. These results may suggest that, besides negatively charged O donors, such as COO^-, alcoholic–O^- and phenolic–O^-, in the event of a favourable steric arrangement the amino group can also participate in binding to Al(III) [39].

As the number of potential binding donors is higher in the aminopolycarboxylates [e.g. iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA)] and polyaminopolycarboxylates [e.g. ethylendiaminetetraacetic acid (EDTA), etc.], they are more efficient Al(III) binders [46,49–53]. In consequence of the large number of coordinating donors, these ligands, with the exception of the tridentate IDA [49,50], form only 1:1



Scheme 1.

complexes. Coordination of the N-donor groups, in addition to the carboxylate functions has been demonstrated by X-ray crystallography [54] and NMR methods [46,49,51]. Interestingly, when one of the carboxymethylene arms of NTA is displaced by a hydroxyethylene function, in $N(CH_2COOH)_2$-(CH_2CH_2OH), dinuclear complexes predominate in a wide pH range, in which monomeric units with IDA-type tridentate coordination are linked together through μ-alkoxide bridges [55,56].

The previous section revealed that primary phosphates are fairly strong binders of hard metal ions, and thus the presence of a phosphate group in the molecule can enhance the Al(III)-binding ability of biomolecules, among them amino acids. The alcoholic–OH or phenolic–OH side-chain groups of amino acids in Ser, Thr or Tyr-containing peptides can be easily phosphorylated and these derivatives occur in biological systems. It has been found, for example, that the neurofibrillary tangles observed in the neurons of Alzheimer's disease patients are especially rich in abnormally and overphosphorylated proteins [57,58]. As the phosphate groups of these proteins are fairly basic, with $pK_a \sim 6$–7, they can bind Al(III) and other hard metal ions quite strongly [59]. As concerns the metal-binding abilities of the building blocks of these proteins, the phosphorylated amino acids, a comparison can be made between the potential binding sites. As discussed earlier, the equilibrium constants characteristic of the Al(III)–amino acid interactions are $\log K \sim 5.9$–6.2, while that for the Al(III)–phosphate interaction is $\log K = 6.1$, i.e. they are comparable, and hence it is reasonable to assume that with hard metal ions the phosphate moiety is a competitive binder with the aminocarboxylate chelating site. In order to demonstrate this and characterise the binding strength quantitatively, we recently studied the interactions between Al(III) and the phosphorylated derivatives of Ser (Ser(P)) and Tyr (Tyr(P)) [60]. It was interesting to observe that Ser(P) was able to keep Al(III) in solution, preventing precipitation even at $pH \sim 8$, while with Tyr(P) precipitation (presumably $Al(OH)_3$) occurred at $pH \sim 5$. Parallel pH-metric and ^{31}P-NMR monitoring of complex formation pointed to the most probable binding models of the various complexes (Fig. 2). Ser(P) is assumed to bind Al(III) both in a monodentate way, via (OPO_3^{2-}) coordination and in a tridentate way, through (OPO_3^{2-}, NH_2, COO^-) chelation. At $pH > 6$ mixed hydroxo complexes $[AlL(OH)]^-$ and $[AlL(OH)_2]^{2-}$ are also formed. Due to the presence of the aromatic ring in Tyr(P), the phosphate group and the aminocarboxylate function are well separated within the molecule and the simultaneous coordination of all three groups cannot occur. This might be the reason for the 'early precipitation' in the Al(III)–Tyr(P) system.

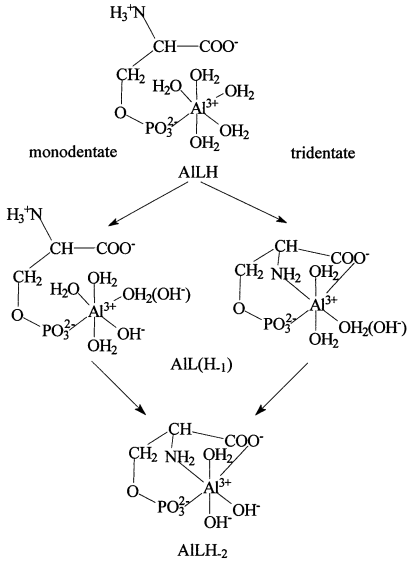


Fig. 2. Binding modes of the complexes formed in the Al(III)–Ser(P) systems.

The ^{31}P-NMR spectra of the Al(III)–Ser(P) system in the pH range 2–4 consist of three peaks: one is that of the free ligand, while the other two, at $\delta = -4.1/-2.9$ ppm and at $\delta = -9.4$ ppm are assigned to the species [Al(LH)(OH)] and [AlL], respectively [60]. The simultaneous observation of these two species in principle allows the determination of the microconstants relative to the formation of [AlL] and [Al(LH)(OH)], which are not distinguishable by potentiometric measurements. At pH 6, only weight-averaged peaks of the free ligand and the complexes [Al(LH)(OH)] and $[AlL(OH)_2]^-$ are observed. This is explained by the acceleration of the ligand exchange reaction due to the presence of OH^- in the coordination sphere.

The stability constant determinations for these simple biomolecules reveal that, although the monodentate or bidentate coordination of one or two such functions is not sufficient to keep Al(III) in solution and to prevent pecipitation of $Al(OH)_3$ at physiological pH, three donor groups in favourable spatial arrangements, even in small biomolecules (such as Asp, Ser(P), etc.), can do this. Let us now consider the Al(III)-binding ability of the larger biomolecules, the peptides.

The interactions of metal ions with oligopeptides are highly influenced not only by the nature (basicity, charge, etc.) of the C and N terminal donors, but also by the presence and relative positions of suitable side-chain donors, which may be able to 'anchor' the metal ions [61]. In the case of oligopeptides consisting of only a few amino acids, the metal ion-induced deprotonation and subsequent coordination of the peptide-NH group play a crucial role in the efficiency of metal binding. For larger peptides or proteins, the suitable spatial arrangement, i.e. some degree of preorganisation of the appropriate donor groups, becomes far more important

in metal binding, and the possibility of amide coordination is generally much more subordinate. For Al(III), no metal ion-promoted deprotonation of the amide has been detected so far in aqueous solution, although, on the basis of FAB MS spectrometric measurements of the adducts formed between oligopeptides (consisting of 2–6 amino acids) and Al(III)-glycerol, Saraswathi and Miller [62] concluded that the primary site of metal ion interaction is the amide N. The reason for the strongly hindered amide coordination observed with Al(III) is in part the lack of suitable anchoring donors in the peptide chain, and in part the very hard character of Al(III) as compared with the much more borderline character of the amide group. Accordingly, the appropriate arrangement of the donors, i.e. the necessary conformation of the peptide/protein molecule, has a decisive role in Al(III) binding. The extent of preorganisation of a peptide, of course, may increase with the molecular weight as the possibility of weak interactions within the molecule increases. A brief review of the interactions of Al(III) with oligopeptides and a detailed discussion of such interactions with a heptapeptide, AcLysSerProValValGluGly, are presented in another paper in this issue.

4. Complexes with nucleotides and other organic biophosphates

Exhaustive reviews on this field have been published in the recent past by Kiss et al. [63] and Nelson [64]. Accordingly, we present here only a brief characterisation of these important interactions and survey in more detail only the papers published since 1995.

Nucleotides contain three different metal-binding sites: phosphate groups in the mono-, di- or triphosphate moieties, alcoholic hydroxy groups in the sugar unit and a carbonyl-O or ring-N in the nucleic base functions. The phosphate binding site can be weakly basic, e.g. the diester in the nucleic acids, DNA and RNA, or it can be basic, e.g. the terminal phosphate in nucleoside phosphates and in many other biophosphates. The basic terminal phosphate ($pK \sim 6$) is the primary binding site for Al(III) [65]. Although some papers indicate the involvement of the base donors in the coordination through macrochelate formation [65–67], most authors rule out any significant interactions of Al(III) with other than the phosphate site [7,63,68]. In contrast with nucleotides, nucleic acids contain only weakly basic phosphates ($pK < 2$), whose metal-binding ability is rather weak; they bind Al(III) at least 10^5 times less strongly than nucleotides. It is quite obvious, therefore, that DNA cannot compete with ATP or other nucleotides or biophosphates for Al(III) [1b].

An LFER relationship was established [23] for the Al(III) complexes of a series of organophosphates, including numerous nucleoside monophosphates (adenosine 5′-monophosphate: AMP, cytidine 5′-monophosphate: CMP, guanosine 5′-monophosphate: GMP, thymidine 5′-monophosphate: TMP and uridine 5′-monophosphate: UMP and also, in order to extend the pK_a range, with phosphonates such as methyl (MP) and ethyl phosphonates (EP) and organic phosphates such as phenylphosphate (PhP) and 4-nitrophenylphosphate (NPhP). The complexes formed with ribose 5′-monophosphate (RibMP) yield information on the possible intervention of the nucleotide base moieties in complexation to the Al(III) ion. The existence of the LFER relationship (the stability constants of the complexes AlL, together with the protonation constants of the coordinating phosphates, are listed in Table 2) proves the same phosphate coordination for all ligands studied, i.e. the exclusion of the nucleotide moieties from the metal ion binding, and also the similar coordination features of the simple phosphonates and phosphates.

Glucose-6-phosphate (G6P), the meeting point of the glucide metabolism for the higher organisms, is present in cells at a non-negligible level, whereas the level of free glucose is low. The two successive pK_a values of G6P are 1.0 and 6.01 [69]. The latter value is comparable to those obtained for other monophosphates (see Table 1). The complexation study of Al(III) with G6P revealed the formation of various mono- and dinuclear complexes: $[AlLH]^{2+}$, $[AlL]^{+}$, $[AlL_2H]$, $[AlL_2]^{-}$, $[AlLH_{-3}]^{-}$ and $[Al_2L_2H_{-n}]^{(2-n)+}$ with $n = 1$–4 (LH_2 is the fully protonated ligand). The stability constant for the formation of the complex $[AlL]^{+}$ ($\log K = 5.60$) is close to that measured for RibMP or nucleoside-5′-monophosphate (Table 2). The ^{31}P-NMR spectra

Table 2
Acidity constants ($-\log K$) and Al(III) stability constants ($\log K$(ML))

	NPhP	PhP	RibMP	MeP	EtP	GMP	UMP	TMP	AMP	CMP
$-\log K(PO_3H_2)$	<1	<1	~1	2.13	2.23	–	–	–	–	–
$-\log K(PO_3H^-)$	5.01	5.76	6.10	7.43	7.65	6.12	6.05	6.24	6.08	6.11
$\log K$(AlL)	4.80	5.29	5.63	6.48	6.63	5.49	5.49	5.66	5.51[a]	5.34[a]

Complexes of various phosphates and phosphonates at 25 °C and $I = 0.2$ M (KCl), data are taken from Ref. [23]
[a] Estimated values.

clearly indicate that the complexation is already effective at pH 1. This allowed us to assume the formation of protonated complexes at acidic pH values, which cannot be easily determined by pH-potentiometry. ^{31}P- and ^{27}Al-NMR studies confirm the presence of the different species in solution. In the ^{31}P-NMR spectra, the signals of the complexes $[AlLH_2]^{3+}$ and $[AlLH]^{2+}$ appear at -7 and -8 ppm (with respect to 85% H_3PO_4 in D_2O), $[AlL]^{+}$ gives a signal at -11.6 ppm, $[AlL_2]^{-}$ at -9 and -11 ppm, $[AlL_2H]$ at -12.9 and -16.8 ppm (two peaks are observed corresponding to one bidentate and one monodentate ligand), $[Al_2L_2H_{-1}]^{+}$ at -4.5 ppm and $[Al_2L_2H_{-2}]$ at around -3 ppm. The multiplicity of the peaks observed for some species is explained by the existence of isomers, resulting from the fact that the sugar ring in the bound ligand can be in different relative positions in the complexes. Essentially three bands are detected in the ^{27}Al-NMR spectra, in accordance with the importance of the corresponding species in the distribution diagram. The peak at -3.5 ppm (with respect to $[Al(H_2O)_6]^{3+}$) can be assigned to $[AlLH]^{2+}$, that at -7 ppm to the complex $[AlL_2]^{-}$ (and $[AlL_2H]$) and that at -1 ppm to $[Al_2L_2H_{-1}]^{+}$. It has been shown that, for the species $[Al_2L_2H_{-n}]^{(2-n)+}$, the $[Al_2L_2]^{2+}$ coordination sphere is completed by hydroxide ions and not by deprotonated hydroxylic functions of the sugar unit; this was demonstrated by ^{13}C-NMR and optical rotation measurements.

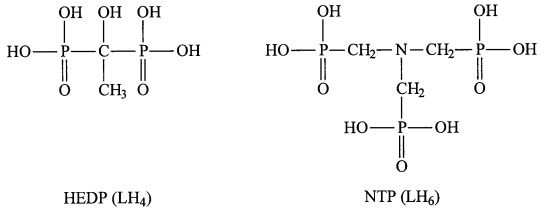
5. Complexes with phosphonates

Phosphonates are clearly distinguished from phopshates by their direct C–P bonds. Many of them, and especially their hydroxo and amino derivatives, have biological activity or can serve as sequestering ligands in metal ion removal, both in biology and in industry. Their metal-binding abilities were recently reviewed [70,71], and the reader is referred to these reviews for more detailed information.

Phosphonate derivatives are used in washing powder formulations. The complexation of Al(III) (and also Cr(III) and Fe(III)) with 1-hydroxyethane-1,1′-diphosphonic acid (HEDP) and nitrilo-tris(methylenephosphonic) acid (NTP) (see Scheme 2) was recently reported [72].

With HEDP, the following Al(III) complexes have been identified: $[AlLH_2]^{+}$, $[AlLH]$, $[AlL]^{-}$, $[Al_2LH]^{3+}$, $[AlL_2]^{5-}$, $[AlL_2H_2]^{3-}$ and $[AlLH_{-1}]^{2-}$. This last complex is the predominant species from pH 5 to 9 for a ligand to metal ratio of 2:1. The ligand is coordinated in a tridentate way through the bisphosphonate moiety and the deprotonated alcoholate function. Formation of a precipitate, identified as $[AlLH_3]$, has been observed in the Al(III)–NTP system.

Al(III) complexation by mixed carboxylate–phosphonate and pure phosphonate derivatives of IDA and NTA [*N*-(phosphonomethyl)glycine (IDmP), iminobis(methyl-phosphonic acid) (IDP), *N*-(phosphonomethyl)iminodiacetic acid (NTmP), *N*,*N*-bis(phosphonomethyl)glycine (NTdP) and nitrilo-tris-(methylphosphonic acid) (NTP)] has been studied by pH-potentiometry and ^{1}H- and ^{31}P-NMR spectroscopy [73]. As a general feature, it was found that substitution of COO^{-} by PO_3^{2-} increases the stability of the complexes in consequence of the higher basicity of the phosphonic groups. However, the higher spatial requirements of the phosphonic function and the greater electrostatic repulsion overcompensate this effect. IDA and its derivatives were found to coordinate in a tridentate way, allowing the formation of bis complexes too, while NTA and its derivatives bind in a tetradentate manner, forming only mono complexes. When the stability constants of the complexes [AlL] are depicted as a function of the number of methylphosphonic arms present in the molecule (see Fig. 3), the IDA derivatives exhibit a nearly linear increase, in accordance with the increasing basicity of the coordinating donors. However, the stabilities of the di- and triphosphono derivatives of NTA differ only slightly, indicating that not all of the phosphonate arms are bound to the metal ion in NTP, because of the high electrostatic repulsion between the phosphonic moieties with their charges of -2. No dinuclear complexes could be detected at a millimolar concentration level, although phosphonate-bridged oligomers were revealed by NMR in solution



Scheme 2.

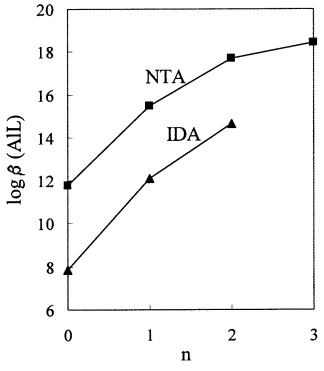


Fig. 3. Stability constants of the AlL complexes of the various phosphonic acid derivatives of IDA and NTA as a funtion of the number of methylphosphonic moieties in the molecules.

[74] and were isolated in the solid state [75]. Interestingly enough, the NMR spectra of Al(III)–NTA and its phosphonic derivatives suggest a very symmetrical donor atom arrangement around the metal ion, though this cannot be achieved because of sterical restrictions. This indicates fast (on the NMR time scale) intramolecular internal motions of the carboxymethylene or phosphonomethylene arms in non-equal chemical environment, resulting in averaged signals corresponding to a fluxional molecule.

6. Complexes with hydroxamates

Siderophores are low molecular mass iron transport and storage molecules in microorganisms. Most of them contain either hydroxamate or catecholate groups and form very stable complexes not only with Fe(III), but with most hard metal ions, including Al(III) [76]. A series of monohydroxamic acids, aceto- (Aha) [77], propano- (Pha), hexano- (Hha), benzo- (Bha), *N*-methylaceto- (MAha), *N*-phenylacetohydroxamic acid (PhAha) and 2-hydroxypyridine-*N*-oxide (Pyrha) [78] have been studied by pH-potentiometry, spectrophotometry and NMR. Besides the normal, hydroxamate (=O, O^-)-coordinated $[AlL_n]^{(3-n)+}$ ($n = 1–3$)-type complexes, mononuclear mixed hydroxo species $[AlL_2(OH)]$ and $[AlL_2(OH)_2]^-$ were detected and identified by ^{27}Al-NMR measurements. The resonance at $\delta = 36.7$ ppm (with respect to $[Al(H_2O)_6]^{3+}$) was assigned to $[AlL_3]$ and the very broad band ($v_{1/2} > 1000$ Hz) at $\delta \sim 56$ ppm to the mixed hydroxocomplex, $[AlL_2(OH)]$. Additional monodentate functional groups, such as amino [77] or 2-hydroxylamino [79], do not affect the basic binding mode of this ligand group, which remains chelated through the hydroxamate O atoms. Stability data (proton and Al(III) complexes) of several aminohydroxamate derivatives are listed in Table 3.

For electrostatic and steric reasons, the presence of such functional groups prevents the formation of tris-complexes. It is interesting that the farther these groups are situated from the hydroxamate function, the stronger the metal-binding ability of the ligand. Accordingly, β-Ala-ha forms stronger complexes than α-Ala-ha, and Glu-γ-ha is a stronger binder than Asp-β-ha. When the additional functional groups are in chelating positions, as in 3,4-dihydroxy-phenyl-hydroxamic acid (DOPA-ha), this extra binding site becomes competitive, and the ligand displays a real ambidentate character. In the acidic pH range, Al(III) binds at the hydroxamate function, where the proton competition is less, while in the basic pH range, at pH > 9, when proton competition is lower at the catecholate function, Al(III) is transferred to this site. In the pH range between 5 and 8, a precipitate is formed, which is presumably a chain-like oligonuclear complex involving a mixed binding mode, with metal ion coordinating at both ends of the molecule [80].

Table 3
Stability constants (log β values) of the proton- and Al(III) complexes of various hydroxamic acids at $t = 25$ °C and at $I = 0.2$ M (KCl)

Species	α–Ala–ha	β–Ala–ha	Asp–β–ha	Glu–γ–ha
HL	9.16	9.42	9.42	9.50
H_2L	16.50	17.91	17.71	18.05
H_3L	–	–	19.83	20.26
AlLH[a]	14.35	19.95	16.27	16.65
Al_2L_2	22.21	–	–	–
$Al_2L_2H_{-1}$	17.59	–	–	–
$Al_2L_2H_{-2}$	12.63	–	–	–
$Al_2L_2H_{-3}$	5.85	–	–	–
$Al_2L_2H_{-4}$	−2.44	–	–	–
AlL_2H_2	–	32.07	31.76	32.79
AlL_2H	–	27.04	26.80	27.62
AlL_2	16.7	19.87	19.26	19.89
AlL_2H_{-1}	9.62	10.74	10.36	9.9
AlL_2H_{-2}	−0.16	0.04	–	1.10

Data from [77].
[a] Charges omitted.

The complexation of Al(III) with desferrioxamine B (DFO), a natural trihydroxamate $[(NH_3^+–](CH_2)_5–NOH–CO–(CH_2)_2–CO–NH]_2–(CH_2)_5–NOH–CO–CH_3]$, is stronger than that observed with any of the monohydroxamates. The ligand H_4L^+ ($pK_1 = 8.30$, $pK_2 = 9.00$, $pK_3 = 9.46$ and $pK_4 = 10.84$ [81]) forms the following Al(III) complexes: $[AlLH_2]^{2+}$ ($\log \beta_{112} = 36.6$), $[AlLH]^+$ ($\log \beta_{111} = 33.8$) and [AlL] ($\log \beta_{11} = 23.9$). In $[AlLH_2]^{2+}$ and $[AlLH]^+$, the Al(III) is bis- and tris-chelated by two and three hydroxamate moieties, respectively, of the same molecule. The transition of $[AlLH]^+$ to [AlL] corresponds to proton release from the non-coordinating ammonium group. The stability data reported by Berthon and coworkers [82] for the biologically relevant 37 °C confirm this speciation model.

7. Aromatic carboxylic and hydroxycarboxylic ligands

Aromatic carboxylic and phenolic compounds can mimic the Al(III)-binding ability of the rather complicated high molecular mass, fulvic and humic acids, present in soil. These functional groups may be of importance in the binding of Al(III) in microorganisms (catecholate-based siderophores) or in plants (e.g. tea).

Because of the high basicity of the donor groups, salicylates ($\Sigma pK \sim 17$) and especially catecholates ($\Sigma pK \sim 22$) chelate Al(III) through the two negatively charged O donors with high stabilities. The binding strength, however, is reduced considerably by proton

competition in neutral aqueous solution [7]. A comparative ^{27}Al- and ^{13}C-NMR study of the Al(III)–phthalic acid (PA), –salicylic acid (SA) and –tiron (TR) systems revealed that the binding constants at pH ~ 3 obeyed the sequence TR > SA > PA, indicating that the stabilities of the complexes depend on the chelate ring size in the order of 5 > 6 ≫ 7-membered ring [83].

Salicylic acid and its derivatives form mono- and bis-chelates; the latter looses two protons above pH ~ 6, resulting in the mixed hydroxo complexes $[AlL_2(OH)]^{2-}$ and $[AlL_2(OH)_2]^{3-}$ [84–88]. In spite of some indications [89,90], it does not readily form an octahedral tris complex since the binding strength of a third salicylate chelate is not competitive enough to suppress metal ion and complex hydrolysis, or even the precipitation of $Al(OH)_3$ at pH > 7. An ^{27}Al-NMR study of the hydrolysis of Al(III) in the presence of salicylate demonstrated that formation of the $[Al_{13}(OH)_{32}]^{7+}$ hydroxo tridecamer is hindered at a ligand to metal ratio higher than 0.5, but colloids are produced above pH ~ 4.5. The 1:1 complex is detected at ~3 ppm (downfield from the signal due to $[Al(H_2O)_6]^{3+}$) [91,92]. The signal corresponding to the complex $[AlL_2]^-$ is assumed to be too broad to be detected. The rate of exchange of water molecules on the Al(III) ion is increased by over three orders of magnitude when salicylate or sulfosalicylate ligands are present in the inner coordination sphere (^{17}O-NMR study [92]). There is apparently a correlation between the exchange rate and the ligand basicity. The potentiometric speciation study by Di Marco et al. [93] with two structurally similar ligands, 2-hydroxyphenylethanone and 2-hydroxybenzeneacetic acid, revealed very similar Al(III)-binding ability with that of salicylate.

In contrast with salicylates, catechol derivatives have much higher affinity for Al(III) in the basic pH range, where the precipitation of $Al(OH)_3$ is prevented by formation of the octahedral tris complex AlL_3 [88,93–97]. The stability of this complex is so high that it can efficiently hinder formation of the very stable tetrahedral hydroxo complex $[Al(OH)_4]^-$, even at pH ~ 12. Oligomeric hydroxo-bridged species are also assumed [88,94] to be present in low concentration in the pH range 5–7. A multinuclear (^{27}Al, ^{13}C and ^{1}H) NMR study of catechol complexation confirmed the pH-metric speciation model [98]. The different resonances observed in the ^{27}Al-NMR spectra were assigned to the following species: the tris chelate at 31.3 ppm (with respect to $[Al(H_2O)_6]^{3+}$), the hydroxo complex $[AlL_2OH]^{2-}$ at between 31.5 and 32 ppm, the bis chelate complex $[AlL_2]^-$ at 26 ppm, and $[AlL]^+$ at 11 ppm (a very broad signal). The bandwidth of the tris chelate signal is relatively low ($\nu_{1/2}$ ~ 340 Hz), revealing the threefold symmetry of this species (species with symmetry lower than octahedral or tetrahedral usually give rise to broad signals; the D_3 symmetry of the catechol tris chelate is not cubic symmetry, but relatively narrow signals are generally observed for this species). At pH > 9.5, new peaks are detected in the range 50–60 ppm; they are assigned to tetrahedral mixed hydroxo complexes. With tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid), the meridional and facial isomers of the chelate $[AlL_3]^{3-}$ (tiron is a unsymmetrical bidentate ligand) were identified in the ^{1}H- and ^{13}C-NMR spectra [99]. In the facial isomer, the ligands are magnetically equivalent; in the meridional isomer, the three ligands are distinct. The meridional to facial ratio was found to be 3:1, as expected from statistical considerations.

Catecholamines that attain significant concentrations in various body fluids, e.g. in the cerebrospinal fluid, can be important Al(III) binders in humans. As a hard metal ion, Al(III) prefers chelation at the negatively charged catecholate locus rather than at the side-chain amino group of catecholamines, and catechol-like binding therefore predominates in a wide pH range. Even for L-DOPA (3,4-dihydroxyphenylalanine), with its chelating glycinate locus, only catecholate coordination occurs [97]. At physiological pH, the main species is an (O^-, O^-)-coordinated tris complex, with ammonium groups remaining protonated.

8. Complexes with aliphatic hydroxycarboxylates

Hydroxycarboxylates, such as lactic acid, malic acid, citric acid, tartaric acid, gluconic acid, saccharic acid, mucic acid, etc., are of special interest in Al(III) biospeciation, as many of them exist in the biological fluids and tissues of the human body and can be low molecular mass binders of Al(III).

Lactate forms weak complexes of $[AlL_n]^{(3-n)+}$ (n = 1–3) type in acidic solution, where the hydroxy group retains its proton while it is coordinated to the metal ion [99,100]. The crystal structure of the tris complex verified that the Al(III) in this complex is surrounded by a distorted octahedral coordination sphere of six O atoms of the carboxylate and the hydroxy groups of three lactate molecules [100,101]. The extents of formation of the bis and tris complexes in solution are rather low, suggesting that the solid compound $[AlL_3]$ does not persist in solution. Towards neutral solutions, $[AlL]^{2+}$ and $[AlL_2]^+$ undergo loss of lactate hydroxy group with pK ~ 4 to give the mononuclear complexes $[Al(H_{-1}L)]^+$ and $[Al(H_{-1}L)L]$. Multinuclear (^{1}H, ^{13}C and ^{27}Al) NMR studies [101,102] suggest the formation of alcoholate-O^--coordinated complexes at neutral pH. However, proton dissociation from a coordinated water molecule and the parallel formation of mixed hydroxo complexes $[Al(OH)L]^+$ and $[Al(OH)L_2]$ can also be assumed. Metal ion-promoted deprotonation and coordinaton of the alcoholic function are more favoured

with malic acid, where the presence of another carboxylate group in the β-position allows the tridentate coordinartion of malate via the formation of a (5 + 6)-membered joint chelate system [103]. Slow reactions produce a variety of polynuclear species containing OH^-, alcoholate-O^- and $-COO^-$ as bridging donors [104]. Such oligomeric species might be important in the high metastability of aqueous solutions of the various Al(III)–hydroxycarboxylate complexes [101].

Citrate [HOOC–CH_2–C(OH)COOH–CH_2–COOH] is widely found in geochemical and biological systems. As a powerful complexing agent, it may solubilise aluminium from clay minerals, permitting the absorption of Al(III) from the environment to the human body. Since it occurs at a concentration of about 0.1 mM in the blood plasma, it can also be a good low molecular mass carrier of the absorbed Al(III) in the blood stream [1b]. For these reasons, citrate is one of the most extensively investigated ligands for Al(III) in aqueous solution. Despite the huge number of speciation studies, however, rather contradictory data have appeared in the literature as concerns the stoichiometry and the stability of the complexes existing at equilibrium [9,48,105–111]. The main reason is the slowness of the oligomerisation reactions taking place in solution. Early potentiometric studies [105,106] led to a simple model involving only the mononuclear 1:1 complexes $[AlLH]^+$, [AlL] and $[AlLH_{-1}]^-$, both in equimolar solution and at an excess of citrate. There is disagreement as regards the proposed binding modes of these mononuclear complexes too. This may not be surprising if the numerous coordination possibilities and protonation states of the donor groups are considered. Citrate has four potential coordination sites: two terminal and one central carboxylate groups and the alkoxy group on the central carbon. Of these potential binding sites, only three can coordinate simultaneously to the same metal ion, resulting in numerous possible structures of the complexes formed. The most likely structure of the complex $[AlLH]^+$ is a tridentate arrangement of one of the terminal carboxylates and the carboxylate and alkoxy groups from the central carbon, with the formation of a (5 + 6)-membered joint chelate system [105]. This latter structure of $[AlLH]^+$ was recently unambiguously proved by means of NMR spectroscopy [48]. The asymmetric character of the AB quartet of the complex (●) (Fig. 4) and the changes in the linewidths of its signals with increasing temperature suggest the above-mentioned binding mode and fluxionality of the complex, i.e. the simultaneous exchange between the bound and the unbound terminal carboxylate groups around the metal ion (Scheme 3). In the species [AlL] and $[AlLH_{-1}]^-$, besides this asymmetric coordination of the ligand molecule, the existence of another coordination isomer involving a (COO_t^-, OH, COO_t^-) chelation was indicated by the NMR spectra [112].

Besides the above-mentioned 1:1 species, bis complexes were found to predominate at a relatively high ligand excess. The proposed complexes are $[AlL_2H]^{2-}$ and $[AlL_2]^{3-}$ in slightly acidic solution, and $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ in neutral and slightly alkaline solution [48,109]. Of these bis complexes, $[AlL_2H_{-2}]^{5-}$ and $[AlL_2H_{-1}]^{4-}$ have recently been isolated as the first mononuclear Al(III)–citrate complexes. Their X-ray structures reveal that the two citrate molecules are bound to Al(III) through one terminal carboxylate and the central carboxylate and hydroxy groups. This tridentate coordination is maintained in solution, and the complexes behave fluxionally, which is reflected in the simple NMR spectra and the broadening of the NMR signals (Fig. 5) [113,114].

It has been found [9,110] that a trinuclear species $[Al_3(LH_{-1})_3(OH)]^{4-}$ (hereinafter $[Al_3L_3H_{-4}]^{4-}$) is formed in a very slow process in slightly acidic solution. This trimeric complex has been crystallised in the form of $[NH_4]_4]Al_3(LH_{-1})_3(OH)(H_2O)]$ from neutral solution, identified unambiguously and characterised in detail by ^{1}H-, ^{13}C- and ^{27}Al-NMR spectroscopy and X-ray crystallography. The complex anion consists of a trimeric Al_3O_4 core, with each citrate coordinated to two or more Al atoms [115]. This very asymmetric structure of the complex persists in solution, resulting in a complicated ^{1}H-NMR signal pattern formed by five AB quartets and a singlet, and in a rich ^{13}C-NMR spectrum consisting of 18 signals [116]. The formation of another trinuclear complex $[Al_3(LH_{-1})_3(OH)_4]^{7-}$, with much more symmetric binding of the citrates, was

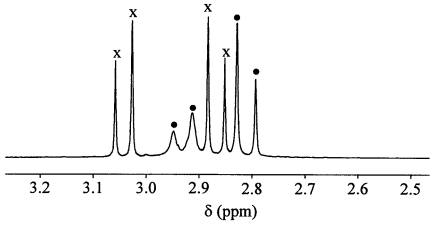
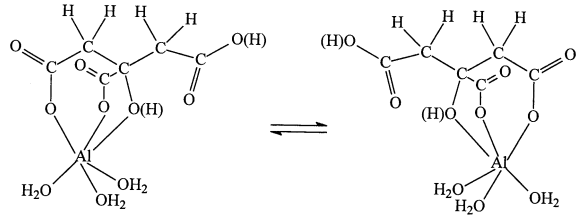


Fig. 4. ^{1}H-NMR spectrum obtained in the Al(III)–citrate system at a ratio 1:1 and pH 2, $c_{Al} = 0.04$ M. Labels: (*x*), free citrate; (●) $[AlLH]^+$ (from [114]).



$[AlLH]^+ - [AlL] - [AlLH_{-1}]^-$

Scheme 3.

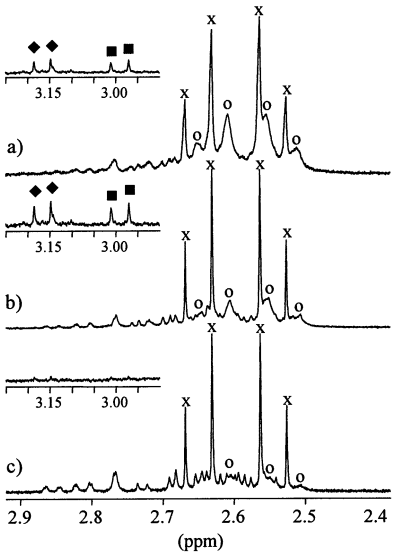


Fig. 5. ^{1}H-NMR spectrum of a 0.07 M solution of $[AlL_2H_{-2}]^{5-}$ in D_2O: (a) 10 min; (b) 3 h; (c) 3 days (at thermodynamic equilibrium) following dissolution of the complex. Labels: (x), free citrate; (■, ◆), citrate complexed in the intermediate species; (○), citrate complexed in the 1:2 complex. In all of the spectra, the region between 3.0 and 3.6 ppm is shown as an insert (fourfold spectral enlargement) (from [114]).

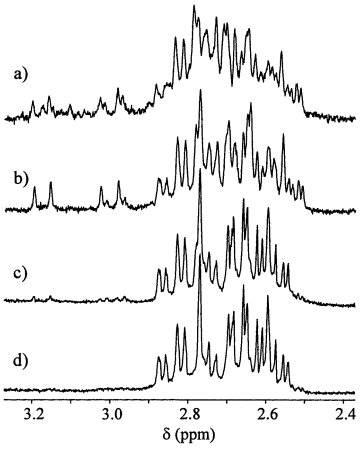


Fig. 6. Time dependence of the ^{1}H-NMR spectra of the Al(III)–citric acid system at ratio 1:1, $c_{Al} = 0.04$ M at starting pH ~ 5. (a) 15 min; (b) 40 min; (c) 10 h; (d) 30 h after mixing of the components.

also suggested at pH > 8 [9]. Detailed time-dependent speciaton studies [9,48] revealed that the mononuclear 1:1 and 1:2 complexes produced in fairly fast reactions react to form oligonuclear species. In the equilibrium state, the trinuclear species $[Al_3L_3H_{-4}]^{4-}$ predominates, even at a ligand excess. The oligomerisation reactions of the mononuclear complexes were followed by ^{1}H-, ^{13}C- and ^{27}Al-NMR spectroscopy too [48,112–114]. Fig. 6 clearly shows the time-dependent changes of the ^{1}H-NMR spectra at pH ~ 5 and a metal ion to ligand ratio of 1:1, and the appearance of the characteristic ^{1}H-NMR pattern of the trinuclear species at equilibrium. The signals observed at lower fields were attributed to the intermediate species formed during oligomerisation [48]. Dissolution experiments on the crystalline bis complexes $[AlL_2H_{-1}]^{4-}$ and $[AlL_2H_{-2}]^{5-}$ indicate their partial decomposition and oligomerisation, finally yielding the trinuclear species [113,114], which represents the thermodynamically stable form. The significant changes in their ^{1}H-NMR spectra over time include the following features (Fig. 5): (i) the AB quartet of free citrate (x) arises immediately upon dissolution of the complex and subsequently increases in intensity; (ii) the intensities of the resonances belonging to the bis complex (o) gradually decrease; and (iii) the rather complicated pattern of the signals of the trinuclear complex emerges after about 3 days. These observations can be explained by the following overall reaction scheme (written here for the complex $[AlL_2H_{-2}]^{5-}$):

$$[Al(LH_{-1})_2]^{5-} + H_2O \rightleftharpoons [Al(LH_{-1})(OH)]^{2-} + L^{3-}$$

$$3[Al(LH_{-1})(OH)]^{2-} \rightleftharpoons [Al_3(LH_{-1})_3(OH)]^{4-} + 2OH^-$$

Obviously, this latter reaction takes place through the formation of an intermediate species, as indicated by the presence of the two doublets at lower fields (■, ◆) in the ^{1}H-NMR spectra, which reach a maximum intensity in time and are not observed in the spectrum obtained at equilibrium (Fig. 5) [114]. The intermediate complex was assigned as a dimer, with an asymmetric coordination of the citrate molecules, as indicated by two-dimensional homo- and heterocorrelated measurements [112,114].

These time-dependent speciation studies reveal that, under model conditions, i.e. millimolar concentrations, the predominant species in the Al(III)–citrate system at equilibrium in the pH-range 3–8 is the trinuclear complex $[Al_3L_3H_{-4}]^{4-}$. In biological fluids, however, this oligomeric species is unlikely to be formed because, due to the continuous metabolism, biological fluids are open systems and never attain real thermodynamic equilibrium. Modelling calculations made at blood serum Al(III) concentrations demonstrate this [48]. The speciation calculations clearly show that at a μM Al(III) level and at pH 7.4, the main species is the mononuclear 1:1 complex $[AlLH_{-2}]^{2-}$. The oligomerisation processes are suppressed. Despite the very high citrate excess as compared to Al(III), the formation of bis complexes in such dilute solutions is also negligible.

The more symmetrical arrangement of the two carboxylic and the two alcoholic–OH groups in tartaric acid makes its coordination somewhat simpler. Potentiometric [117,118] and NMR studies [119,120] have revealed mononuclear (mostly 1:1) and binuclear (2:2) complexes, with both octahedral and tetrahedral Al(III) centres. The ^{13}C and ^{27}Al chemical shifts showed that deprotonation of the Al(III)-coordinated hydroxy

groups of tartaric acid occurs at a pH as low as ~2. In the dinuclear species, the Al(III) ions are bridged by two quadridentate (4-) ligand molecules [118,119]. It is noteworthy that the mononuclear tris complex $[Al(H_{-1}L)_3]^{6-}$ detected by NMR in basic solution seems to be remarkably stable, because of the successive interligand hydrogen bonds between the coordinated and deprotonated alcoholate groups and the non-coordinated alcoholic–OH groups [119,120]. A combined IR and ^{1}H-NMR investigation of the solution state of several commercially available solid-state Al(III) preparations revealed that their solution state generally differs from their formula and they readily form metastable solutions [121,122].

The structurally very similar saccharic acid and its isomer mucic acid form similar complexes: dinuclear 2:2 species with different protonation states predominate in the pH range 3–8 [123]. The stoichiometry and binding mode of the complexes were established by means of ESI-MS and multinuclear 2D NMR measurements. Interestingly, the ligands seem to coordinate in a non-symmetrical way. While one end of the bridging ligand molecule coordinates in a tridentate manner (COO^- and two (de)protonated alcoholic–OH groups), the other end binds in a monodentate way (COO^-). A similar binding mode of saccharic acid is observed in some of its Al(III) and alkaline earth ternary metal complexes [124].

9. Biological implications

A knowledge of the metal-binding abilities of biologically relevant endogenous or exogenous bioligands and a quantitative solution speciation characterisation of such interactions is currently finding increasing biological use. Such information can help in a determination of the speciation of metal ions bound to the low molecular mass constituents of biological fluids and tissues through computer simulation, provided that all the total or free concentrations of the constituents of a given biofluid, and the formation constants of all the complex species into which they are distributed, are known. The problems, difficulties and recent achievements of this approach are discussed in some detail in the paper by Berthon et al. in this issue [125]. Here, we present only a few examples on how these results can be utilised to provide an estimation of the mobility of Al(III) in biosystems, including its transport and bioavailability.

A speciation description of Al(III) under blood serum conditions could be important because of the different results and opinions to be found in the literature as concerns the most important low molecular mass Al(III) binders in the blood plasma. Inorganic phosphate [126–128] and/or citrate [16,129,130] have been suggested as the main Al(III) binders. The formation of ternary Al(III)–citrate–phosphate complexes is usually ignored, in spite of the fact that both ligands are present simultaneously in the serum. Our recent time-dependent speciation study of the Al(III)–citrate(L)–phosphate(B) system [131] revealed the formation of four ternary complexes: $[AlLBH_2]^-$, $[AlLBH]^{2-}$, $[AlLB]^{3-}$ and $[AlLBH_{-1}]^{4-}$. For the first time in the literature, the binary Al(III)–phosphate species were detected directly at pH 7.4 by ^{31}P-NMR spectroscopy. The slow formation of the trinuclear complex $[Al_3L_3H_{-4}]^{4-}$ at the expense of the initially formed binary Al(III)–citrate complexes and ternary species was monitored by NMR.

At the physiological pH 7.4 and at a mM model concentration level of Al(III), most of the metal ion is bound in the ternary complexes $[AlLB]^{3-}$ and $[AlLBH_{-1}]^{4-}$. In contrast, at blood serum concentrations (μM level) ~50% of the non-transferrin-bound Al(III) is present in the form of the binary citrato complex $[AlLH_{-2}]^{2-}$, but the formation of the ternary species and the binary phosphato complex $[AlBH_{-1}]^-$ cannot be neglected (Fig. 7). Citrate-bound Al(III) was observed directly in the blood plasma by Sadler and coworkers [132] by means of ^{1}H-NMR spectroscopy, and by Bantan et al. [133] by using fast protein liquid chromatography. In a more recent study, these latter authors [134] investigated the speciation of low molecular mass Al(III) complexes in human serum taken from healthy volunteers and demonstrated the presence of ternary complexes too. They found that the main such Al(III) species present in the serum were binary Al(III)–citrate, Al(III)–phosphate and ternary Al(III)–citrate–phosphate complexes. The distribution of these complexes varied from individual to individual.

As a continuation of his exhaustive review in this field [24], Berthon et al. give further details in their recent papers [118,135–137] on the potential involve-

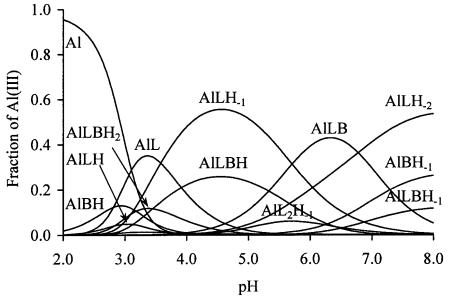


Fig. 7. Species distribution curves for complexes formed in the Al(III)–citrate(L)–phosphate(B) system at blood serum concentrations ($c_{Al} = 6 \times 10^{-7}$ M, $c_{citrate} = 99 \times 10^{-6}$ M, $c_{phosphate} = 1.1 \times 10^{-3}$ M).

ment of small biomolecules in the metabolism of Al(III). Comparing the abilities of dietary acids such as succinic acid [135] and tartaric acid [110] to favour the absorption of Al(III) in the gastrointestinal tract, they found that tartaric acid is much more efficient than succinic acid. Tartaric acid, which forms different neutral complexes over the pH interval 2.5–7.5 [118], can keep Al(III) soluble at a normal concentration level throughout the whole pH range of the small intestine, which is likely to enhance its bioavailability. At the same time, succinic acid is able to facilitate the gastrointestinal absorption of Al(III) only at high concentrations of the metal ion [135,136]. It has been found that the co-occurrence of dietary phosphate reduces the fraction of Al(III) neutralised by tartrate, and thus the absorption of the metal ion falls; however, it is not completely eliminated. In contrast, succinate is not able to increase Al(III) absorption when it is ingested concomitantly with Al(III) and phosphate. It is interesting that plasma simulations suggest that neither tartrate nor succinate can influence the fate of Al(III) in the blood plasma, and thus cannot enhance aluminium excretion [118,135,136].

Although the applicability of chelation therapy in senile dementias is still debated, its value in an Al-overload or acute Al(III) toxicity is well documented [138–140]. Yokel et al. published outstanding reviews [141,142] of the applicability of a wide variety of chemical compounds, including fluoride, carboxylic acids, amino acid derivatives, catechols, polyamino carboxylic acids, phenyl carboxylic acids, the hydroxypyridinones and hydroxamic acids. In a recent paper the Al-mobilising capacities of four ligands [2,3-dihydroxybenzoic acid (DHBA), tiron, 3-hydroxy-1,2-dimethylpyridin-4-one (CP 20) and 3-hydroxy-1,2-diethylpyridin-4-one (CP 94)], possible substitutes to desferrioxamine (DFO) and also of DFO itself, were measured [137]. Plasma mobilising index calculations revealed that at pH 7.4 and under blood plasma conditions, CP 20 and CP 94 exhibit similar Al-mobilising abilities. At pH 6.6 and 5.5 (encountered under inflammatory conditions), both pyridinone derivatives exhibit better mobilizing capacities than that of DFO at concentrations higher than 10^{-3} M. Tiron, which is relatively efficient at pH 7.4, is less efficient at lower pH values (protonation overcomes complexation). DHBA proved to be the least efficient, its Al-mobilizing capacity decreasing strongly with decreasing pH.

The practical limitations of DFO, i.e. the lack of oral efficacy and its occasional side-effects, encourage the search for orally effective substitutes of DFO. A great deal of research is being carried out in this field. The bidentate 3-hydroxypyridin-4-ones are currently the most encouraging alternatives to DFO [142].

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