DALTON

Complexation of aluminium(III) with several bi- and tri-dentate amino acids

Tamás Kiss,*[a] Imre Sóvágó,[b] Imre Tóth,[b] Andrea Lakatos,[a] Roberta Bertani,[c] Andrea Tapparo,[d] Giorgio Bombi[d] and R. Bruce Martin[e]

[a] *Department of Inorganic and Analytical Chemistry, Jozsef Attila University, H-6701, Szeged, PO Box 440, Hungary*
[b] *Department of Inorganic and Analytical Chemistry, Kossuth University, H-4010 Debrecen, PO Box 21, Hungary*
[c] *Centro di Studio sulla Chimica e Tecnologia dei Compositi Metallorganici degli Elementi di Transizione, CNR, Via Marzolo 9, I-35131 Padova, Italy*
[d] *Dipartimento di Chimica Inorganica Metallorganica Analitica, Via Marzolo 1, I-35131 Padova, Italy*
[e] *Department of Chemistry, University of Virginia, Charlottesville, VA 22903, USA*

Complex formation between Al^{III} and various bidentate and potentially tridentate amino acids (Gly, Ser, Thr, Gln, Asn, Glu and Asp) and some model compounds (*N*-acetylaspartic acid, succinic acid, and 2-sulfanylsuccinic acid) was studied in aqueous solution by pH-potentiometric and multinuclear (^{1}H, ^{13}C and ^{27}Al) NMR techniques. A weak interaction through the carboxylate function was unambiguously detected for all of these ligands. For Asp, however, which contains two carboxylates and a central amino donor, tridentate co-ordination is strongly suggested. Although its steric environment is similar to that of the amino function in Asp, binding of the thiolate sulfur of 2-sulfanylsuccinic acid could not be verified.

Aluminium is known to form fairly stable complexes with negatively charged O-donor chelating biomolecules such as aliphatic and aromatic hydroxycarboxylates, catecholates, phosphates, *etc*. Its binding ability to the protein building block α-amino acids, however, has not yet been established unambiguously.[1–5]

The α-carboxylate group of amino acids is weakly basic ($pK \approx 2.2$), which suggests a rather weak aluminium(III) binding capability. The usual sample composition in the pH-potentiometric titration method (a metal-ion concentration of a few mmol dm^{-3} with a metal ion to ligand ratio of from 1:1 to 1:5) is not suitable for the detection of complex formation.[1] 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 was explained in terms of the electrostatic repulsive effect of the NH_3^+ group.[1] Use of an LFER (linear free-energy relation) approach led to a log β value of 5.8 being estimated for Al^{3+} and glycinate.[3] According to this estimate, co-ordination/chelation of Al^{3+} should become distinguishable from hydrolysis at glycine (Gly) concentrations greater than 25 mmol dm^{-3}. Precise pH-metric studies have been carried out by Dayde[4] in several Al^{III}–amino acid (Gly, Ser, Thr, Asp, Glu and His) systems at 37 °C and $I = 0.15$ mol dm^{-3} (NaCl) and at a high ligand concentration of 0.02 mol dm^{-3}. Stability constants for 1:1 complexes were obtained by parallel refinement of the stability constants of the Al^{III}–hydroxo and –amino acid complexes. A log K_{AlA} value in the range 5.7–6.2 was determined for the bidentate amino acids. In contrast, Yadava and co-workers,[2] detected the formation of much stronger AlA, AlA_2 and AlA_3 complexes with Leu, Ser and Thr by using an ionophoretic technique, although admitted the rather limited precision of the method. Rao and Rao[5] recently made a qualitative characterization of the interaction between Al^{3+} and Ala. With potentially tridentate amino dicarboxylic acids such as Asp and Glu a somewhat stronger interaction could be detected: the formation of various protonated species, and ternary dinuclear hydroxo species, was assumed.[4–6]

In the present paper we evaluate the stability constants for the binding of Al^{III} to various amino acids, such as Gly, Ser, Thr, Gln, Asn, Glu and Asp. A comparative study was carried out with *N*-acetylaspartic acid (Ac-Asp), succinic acid (H_2succ) and 2-sulfanylsuccinic acid (H_3Ssucc). The speciation results of the detailed pH-potentiometric measurements were confirmed by ^{1}H, ^{13}C and ^{27}Al NMR measurements.

Experimental

Reagents

The amino acids, the best quality compounds available from Reanal Co., were purified by recrystallization. The compounds H_2succ and H_3Ssucc were Aldrich products. Their purities were checked, and when possible the exact concentrations of their solutions (prepared freshly each day) were determined by the Gran method.[7] The stock solution of Al^{3+} was prepared from recrystallized $AlCl_3 \cdot 6H_2O$ and its metal concentration was determined gravimetrically *via* its quinolin-8-olate. To prevent hydrolysis, the metal-ion stock solution contained 0.1 mol dm^{-3} HCl. The ionic strength of all solutions measured was adjusted to 0.20 mol dm^{-3} KCl. In all cases the temperature was 25.0 ± 0.1 °C.

pH-metric measurements

The stability constants of the proton and aluminium(III) complexes were determined by pH-metric titration of 25.0 cm^3 samples. Owing to the weak complexation reactions, measurements were performed at high total concentrations and at high excesses of the amino acids, 0.02 or 0.04 mol dm^{-3}, and the metal ion to amino acid ratio was 1:10, 1:15, 1:20, 1:25, 1:30 or 1:40. Owing to solubility limitations, Asp was measured at 0.016 mol dm^{-3}. The titrations were performed until precipitation occurred, with carbonate-free KOH solutions of known concentration (*ca*. 0.2 mol dm^{-3}) under a purified argon atmosphere. Depending on the amino acid and the metal ion to

amino acid ratio, precipitation occurred in the range pH 4.5–5.8. The reproducibility of the titration curves was within 0.010 pH units throughout the whole pH range. When equilibration could not be reached in 10 min, titration points were omitted from the calculations.

The pH was measured with a Radiometer PHM 84 instrument with a GK2322C combined glass electrode, which was calibrated for hydrogen-ion concentration according to Irving *et al.*[8] The ionic product of water was found to be $pK_w = 13.76$. The concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated with the aid of the computer program PSEQUAD.[9] For the hydroxo complexes of Al^{III}, the stability constants (log β) assumed[10] were −5.52 for $[AlH_{-1}]^{2+}$, −13.57 for $[Al_3H_{-4}]^{5+}$, −109.1 for $[Al_{13}H_{-32}]^{7+}$ and −23.46 for $[AlH_{-4}]^{-}$.

NMR measurements

Proton, ^{13}C and ^{27}Al NMR spectra were recorded at 25 °C on Bruker WP 200SY and AC200 spectrometers in D_2O. The pD values were calculated *via* the equation pD = pH-meter reading + 0.4.[11] Quantitative ^{27}Al NMR spectra were recorded using the 'absolute integration mode', in order to allow comparison of the integral values obtained for the different samples. Typical NMR parameters were: pulse width = 5 ms (30°), pulse repetition time = 1.262 s.

Results and Discussion

Interactions of Al^{III} with bidentate amino acids (Gly, Ser, Thr, Asn and Gln)

Speciation results. Owing to the expected rather weak interactions of Al^{III} with bidentate aminocarboxylates and its high hydrolytic tendency, very high amino acid excesses were applied in the titration samples. Titration curves of the acid alone, Al^{3+} alone and the Al^{3+}–Gly sample at a 1:15 metal ion to acid ratio are depicted in Fig. 1. These curves indicate that the pH range of metal–ligand complexation is well separated from the deprotonation (buffering) pH range of the amino acid, and thus the buffering effect of the large excess of the latter is negligible in this pH range (see curves 1 and 2). At the same time the overlap with the hydrolytic processes of Al^{III} is considerable (see curves 1 and 3). The small, but consequent difference between curves 2 and 3, however, can be unambiguously ascribed to the proton-displacement reactions of Al^{III}, *i.e.* to the reaction $Al^{3+} + HA \rightleftharpoons AlA + H^+$. Accordingly, the metal–ligand formation constants can be determined with acceptable precision.[12]

Titration curves obtained at high amino acid excess were evaluated by assuming different equilibrium models. The best fit between the experimental and the calculated titration curves was obtained with the species and stability constants listed in Table 1. The pH ranges included in the calculation are also given. It is seen that these Al^{III}–amino acid systems can be fairly well described by the same speciation model, assuming a 1:1 complex $[AlA]^{2+}$, its deprotonated form $[AlAH_{-1}]^{+}$, and the dinuclear species $[Al_2AH_{-1}]^{4+}$. Other complexes, such as $[Al(HA)]^{3+}$, and all 1:2 complexes, were rejected by the computer program. As an illustration, species distribution curves for the Al^{III}–Gly system are depicted in Fig. 2.

Proton, ^{13}C and ^{27}Al NMR measurements. Although the pH-metric results are fairly convincing, independent ^{1}H, ^{13}C and ^{27}Al NMR measurements were also made unambiguously to demonstrate this weak interaction between Al^{III} and simple bidentate amino acids. First, the ^{1}H and ^{13}C NMR spectra of

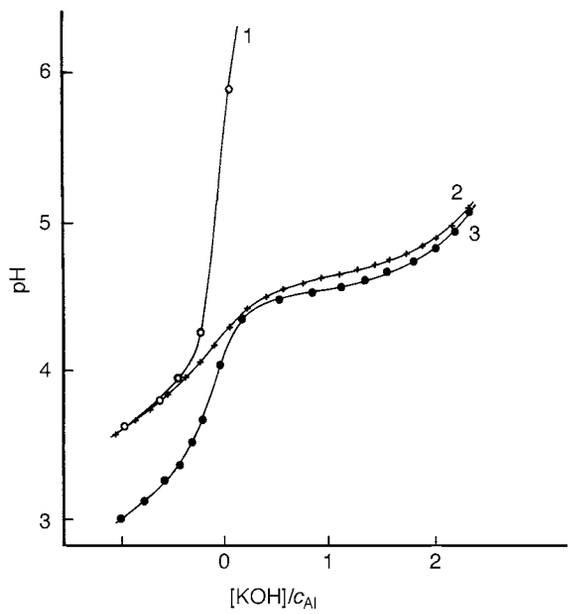


Fig. 1 Titration curves of samples containing Al^{3+} and/or Gly: 1 (○), $c_{Gly} = 0.04$ mol dm^{-3}; 2 (+), $c_{Gly} = 0.04$, $c_{Al} = 0.0027$ mol dm^{-3}; 3 (●), $c_{Al} = 0.0027$ mol dm^{-3}

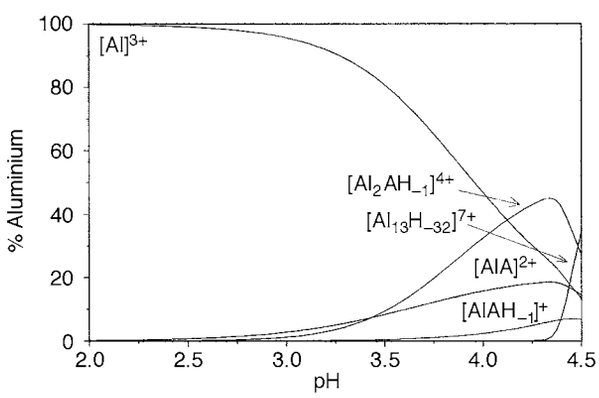


Fig. 2 Species distribution curves in the Al^{III}–Gly system. $c_{Gly} = 0.16$, $c_{Al} = 0.008$ mol dm^{-3}

Table 1 Proton (log *K*) and aluminium(III) complex-formation constants (log β) of several bidentate amino acids at 25 ± 0.01 °C and *I* = 0.20 mol dm^{-3} (KCl)

	Gly	Ser	Thr	Gln	Asn
log $K(NH_2)$	9.57(1)	9.02(1)	8.91(1)	8.99(1)	8.71(1)
log $K(CO_2^-)$	2.34(2)	2.16(2)	2.15(2)	2.16(2)	2.14(2)
$[AlA]^{2+}$	5.91(10)	5.66(11)	5.51(12)	5.61(13)	5.50(28)
$[AlAH_{-1}]^{+}$	1.08(9)	0.62(23)	0.94(15)	1.33(4)	1.31(8)
$[Al_2AH_{-1}]^{4+}$	4.35(9)	3.75(11)	—	—	—
Fitting*	0.0108	0.0109	0.0130	0.0058	0.0134
No. of points	248	160	80	84	168
pH Range	2.6–4.9	2.3–5.4	2.6–4.8	2.3–4.7	2.3–5.2
$Al + HA \rightleftharpoons AlA + H$	−3.66	−3.36	−3.40	−3.38	−3.21
$AlA \rightleftharpoons AlAH_{-1} + H$	−4.83	−5.04	−4.57	−4.28	−4.19

* Average difference in the calculated and experimental titration curves expressed in cm^3 of the titrant.

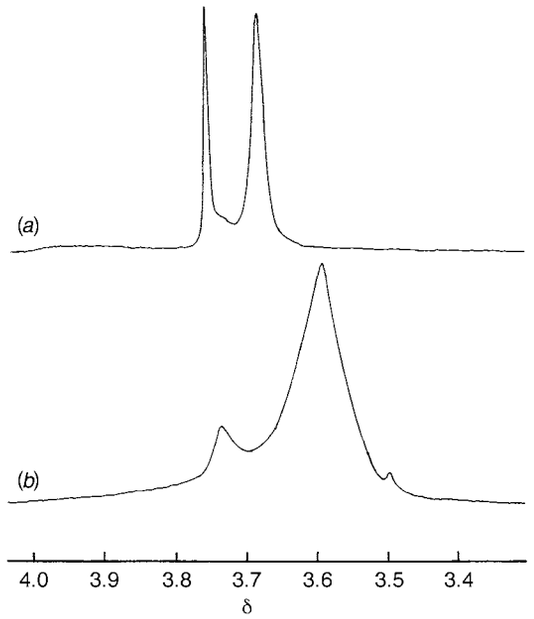


Fig. 3 Proton NMR spectra of the Al^{3+}–Gly system at 2.5 (*a*) and 3.5 pH (*b*); $c_{Al} = 0.5$, $c_{Gly} = 0.5$ mol dm^{-3}

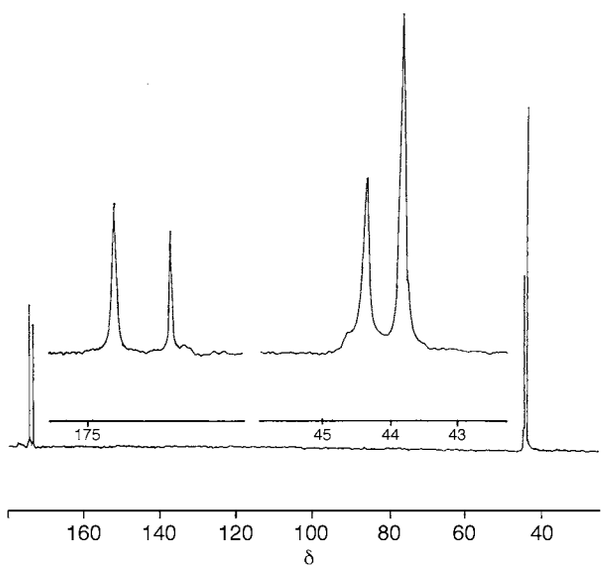


Fig. 4 Carbon-13 NMR spectra of the Al^{3+}–Gly system; $c_{Al} = 0.5$, $c_{Gly} = 0.5$ mol dm^{-3}, pH 2.44

the H–Gly and Al^{III}–Gly systems were taken at high concentrations ($c_{Al} = c_{Gly} = 0.5$ mol dm^{-3}) at pH ≈2.5 (the highest pH before precipitation occurred). The spectra were found to consist of two clearly separated sets of signals: the complexation resulted in a well detectable 0.07 ppm downfield shift in the $^1H(CH_2)$ signal [see Fig. 3(*a*)], while a 0.46 ppm downfield shift in the $^{13}C(CH_2)$ signal and a 1.0 ppm upfield shift in the $^{13}C(CO)$ signals (see Fig. 4). As is seen in Fig. 3(*a*), under these conditions nearly 50% of the total Gly is bound to Al^{III}. With dilution, of course, dissociation of the complex increases, but as shown in Fig. 3(*b*) complex formation can be unambiguously detected even in 0.05 mol dm^{-3} solutions. The separated signals indicate a 'slow exchange' range for the complex and the free amino acid system. Substantial broadening of the signals of the free amino acid is observed, especially at pH 3.5 for the more dilute sample. This cannot be explained by a mutual two-site exchange between the complex and free amino acid, as the equality $p_1(\Delta\nu_{\frac{1}{2}})_1 = p_2(\Delta\nu_{\frac{1}{2}})_2$ (where p is the molar ratio of each site and $\Delta\nu_{\frac{1}{2}}$ is the half width of the original) is not fulfilled. A detailed study of the kinetics of these processes is under progress. At mmol dm^{-3} aluminium(III) concentrations high excesses of amino acid have to be applied to enhance the Al^{III}–amino acid interaction, and thus complex formation cannot be monitored using the ligand nuclei; accordingly ^{27}Al NMR measurements were carried out.

Table 2 Aluminium-27 NMR spectral data for Al^{III}–amino acid interaction

c_{Al}/mol dm^{-3}	pH	δ	$\Delta\nu_{\frac{1}{2}}$/Hz	Area
Al^{3+} solution				
0.008	1.26	0.50	4	100
0.008	4.08	0.71	50	104
0.008	4.51	1.14	100	60
		63.6	12	10
Al^{III}–Gly 1:20 ratio				
0.008	3.09	0.55	6	100
0.008	4.08	0.96	79	98
0.008	4.49	1.20	120	40
		63.6	24	2
0.008	5.02	63.5	40	4
Al^{III}–Ser 1:20 ratio				
0.008	3.01	0.50	5	110
0.008	3.96	0.86	73	100
0.008	4.47	0.93	144	41
		63.7	26	3
0.008	5.00	63.7	35	4
Al^{III}–Asp 1:5 ratio				
0.003	4.08	0.93	90	36
		9.9	130	29
0.003	4.47	1.37	120	12
		9.9	130	29
0.003	5.02	10.0	180	14
Al^{III}–H_3Ssucc 1:5 ratio				
0.003	3.53	0.73	44	60
0.003	4.02	0.90	110	25
0.003	4.53	1.70	140	Precipitate

Since Al^{III} is quadrupolar, asymmetry in the ligand field produces an increase in the NMR linewidth. The resonances of low-symmetry aluminium(III) species can be so broad (linewidth higher than a few thousand Hz) that they cannot be detected in practice as they merge into the baseline. When the symmetry is high, *e.g.* in $[Al(H_2O)_6]^{3+}$, the ^{27}Al spectral peak is sharp: in an 8×10^{-3} mol dm^3 Al^{3+} solution at pH 1.26 a single peak with a linewidth of 4 Hz was measured. For the same solution at pH 4.08 a single peak still occurred at δ 0.71, but with a much larger linewidth, while at pH 4.51 three peaks could be detected: at δ 1.14 ($\Delta\nu_{\frac{1}{2}} = 100$), at 63.6 (12) and a very broad band ($\Delta\nu_{\frac{1}{2}} > 1000$ Hz) centred around δ 30. The peak at δ ≈1 relates to Al^{III} in a fairly symmetrical octahedral environment; the other sharp signal, at around δ 63.6, can be ascribed to the central tetrahedral Al^{III} of the tridecanuclear hydroxo species,[13] while the remainder of the Al^{III}, in a much less symmetrical environment, is reflected by the broad band. Area integration revealed that in this sample ≈60% of the Al is involved in peak 1, ≈10% in peak 3 and the rest in peak 2. In a similar way, we used area integration to estimate the amount of Al^{III} found in the different chemical environments in solutions containing a high excess of amino acid (Gly or Ser) besides Al^{III}. The results listed in Table 2 show that at pH ≈3.0 the presence of a high excess of amino acids has practically no effect on the ^{27}Al NMR spectrum: all the Al^{III} is in octahedral species {mostly $[Al(H_2O)_6]^{3+}$}. At pH 4.5, however, in the presence of a 20-fold excess of the amino acids, 20% less Al^{III} is to be found in octahedral environment. At pH ≈5 the solution is still clear [whereas in the absence of ligands $Al(OH)_3$ precipitates from the solution], and only Al^{III} bound in the tridecanuclear species can be clearly detected, but most of the Al^{III}

must be in a less symmetrical environment and this gives a very broad, hardly detectable band.

Comparison of the speciation curves (Fig. 2) and the ^{27}Al NMR data (Table 2) indicates a reasonably good correlation between the two. At pH ≈3.0, where Al^{III} is almost exclusively in the free form, the total aluminium yields a signal at around δ ≈0.5. The somewhat larger linewidth as compared with that of $[Al(H_2O)_6]^{3+}$ under more acidic conditions can be explained by the effect of the relatively slow ligand-exchange reaction. A similar broadening of the signals was observed by Garrison and Crumbliss[14] for the Al^{III}–hydroxamic acid systems. When the pH is increased to ≈4 or ≈4.5 increasing amounts of Al^{III} disappear from the octahedral aluminium(III) band at δ ≈1 as compared with the intensity-distribution data obtained for the ligand-free Al^{3+} solution. Similarly, the relative amount of the tridecanuclear hydroxo species also decreases. The 'missing' Al^{III} is probably bound to amino acid in a fairly unsymmetrical geometry, thereby resulting in a very broad, hardly detectable ^{27}Al NMR band. At pH ≈5.0 only the tridecanuclear species gives a sharp signal in the presence of amino acids. As its relative intensity is somewhat higher than at pH 4.5, OH^- tends to displace simple amino acids from the co-ordination sphere of Al^{III} with increasing pH. The speciation and the multinuclear NMR data unambiguously demonstrate the interaction between Al^{III} and simple amino acids under weakly acidic conditions at the mmol dm^{-3} aluminium(III) level.

What can be said about the bonding mode in the aluminium(III) complexes? As the affinity of Al^{III} for N-donors is very low,[3] it is not too likely that the NH_2 group of these amino acids is involved in aluminium(III) binding. Hence, it is probably in protonated form (NH_3^+) in weakly acidic solutions. Accordingly, $[AlA]^{2+}$, the first complex formed, must be a mixed-ligand hydroxo complex $[Al(HA)(OH)]^{2+}$. The other microform $[AlA]^{2+}$ containing a five-membered chelate may be a minor form. The formation constants obtained for the species are in fairly good agreement with those determined by Dayde.[4] The species $[AlA]^{2+}$ loses a proton with pK ≈4.2–5.0, which should be ascribed either to the ionization of a second water molecule in the co-ordination sphere of Al^{III} or to deprotonation and co-ordination of the amino group. The complex, however, is only a minor species. The fairly low pK_{AlA} values {0.5–1.0 log unit lower than pK for $[Al(H_2O)_6]^{3+}$ = 5.52; see Table 1}, *i.e.* the slight increase in acidity of the water molecules in the complex should indicate either (*i*) involvement of the amino group in the co-ordination (see above), or (*ii*) a change in the co-ordination geometry from octahedral to tetrahedral during the water ionization process. It must be mentioned that a similar acidification of the co-ordinated water molecules was observed in the case of Al^{III}–adenosine 5′-phosphate complexes.[15] It is seen in Fig. 2 that the mononuclear species $[AlA]^{2+}$ is formed in parallel with a dinuclear complex $[Al_2AH_{-1}]^{4+}$ in the same pH range. It was found that these two species could partly replace each other in the calculation. The fitting parameter changed only a little, when besides $[AlAH_{-1}]^+$, only one of them was assumed in the calculation. The stability data listed in Table 1 were obtained when both species were simultaneously included in the speciation model. The ion $[Al_2AH_{-1}]^{4+}$ is very probably a carboxylate- and dihydroxo-bridged complex with a protonated ammonium group, *i.e.* $[Al_2(OH)_2(HA)]^{4+}$. A complex with this binding mode has also been observed in Al^{III}–monocarboxylic acid[16] and –carbonate systems.[17] As this structure is found in minerals containing dihydroxo-bridged aluminium chains, Öhman[16] assumes that it is a generally occurring structure for all dinuclear Al^{III}–monocarboxylato complexes. He demonstrated that the ability of Al^{III} to form this complex increases with increasing pK_{CO_2H} and established approximate linearity between the log K values characterizing the formation reaction $2Al^{3+} + A \rightleftharpoons Al_2AH_{-2} + 2H^+$ and the pK_{CO_2H} values. The above tendency exists for the aminocarboxylates Gly and Ser, as the significantly lower basicity of the carboxylate functions of the amino acids (pK ≈2.1–2.3) results in a lower stability of the dinuclear complexes.

Aluminium(III) interactions with Glu, Asp and some related compounds

Speciation results. Glutamic acid and aspartic acid, containing carboxylic functions (which have a high affinity for Al^{III}) at both ends of the molecule, can be potentially more efficient binders of Al^{III} than the amino acids discussed so far. With transition-metal ions, where the basic bonding mode of α-amino acids is five-membered (CO_2^-, NH_2) chelation, Asp readily acts as a tridentate ligand, with formation of a five- + six-membered (CO_2^-, NH_2, CO_2^-) joint chelate system. The tridentate co-ordinating ability of Glu is much weaker, due to the lower stability of the seven-membered second chelate ring. Thus, Glu rather acts as a bidentate ligand showing similarity with simple amino acids. Aspartic acid (Asp) has been shown to form (CO_2^-, CO_2^-) chelates (*via* formation of a seven-membered ring), especially with hard metal ions.[18] Although Al^{III} has low affinity for N-donors, in Asp, where the amino group is adjacent on both sides to strong Al^{III}-binder carboxylate functions, metal ion-induced deprotonation of the NH_3^+ group and co-ordination of the amino group may be more

Table 3 Proton (log K) and aluminium(III) complex-formation constants (log β) of several tridentate amino acids and related compounds at 25 ± 0.01 °C and I = 0.20 mol dm^{-3} (KCl)

	Glu	Asp	Ac-Asp	H_2succ	H_3Ssucc[a]
log K(NH_2)	9.50(1)	9.62(1)	—	—	10.20(3)
log K(CO_2^-)	4.09(2)	3.66(1)	4.49(2)	5.24(2)	4.47(2)
log K(CO_2^-)	2.04(3)	1.94(5)	3.04(2)	3.96(2)	3.05(2)
$[AlHA]^{2+}$	10.88(22)	11.76(6)	6.15(3)	7.03(15)	12.99(2)
$[AlA]^+$	7.29(4)	7.87(4)	2.24(9)	3.63(8)	8.63(9)
$[AlAH_{-1}]$	2.55(3)	3.30(3)	−1.91(14)	−0.53(5)	4.05(4)
$[AlAH_{-2}]^-$	—	−2.32(7)	—	−5.55(9)	—
$[Al_2A]^{4+}$	9.46(15)	—	—	—	—
Fitting[b]	0.0092	0.0128	0.0075	0.0086	0.0128
No. of points	244	201	158	223	113
pH Range	2.6–5.0	2.5–5.9	2.0–4.6	2.0–5.9	2.5–5.0
$Al + HA \rightleftharpoons Al(HA)$	1.38	2.14	1.66	1.79	2.79
$AlHA \rightleftharpoons AlA + H$	−3.59	−3.89	−3.91	−3.40	−4.36
$AlA \rightleftharpoons AlAH_{-1} + H$	−4.74	−4.57	−4.15	−4.16	−4.58
$AlAH_{-1} \rightleftharpoons AlAH_{-2} + H$	—	−5.62	—	−5.02	—
$Al + H_2A(H) \rightleftharpoons AlA(H) + 2H$	−4.75	−3.46	−5.29	−5.57	−4.73
$Al + H_2A(H) \rightleftharpoons AlA(H)H_{-1} + 3H$	−8.34	−7.35	−9.44	−9.73	−9.09

[a] As the neutral form of H_3Ssucc is H_3A, while that of the other ligands is H_2A the charge of the corresponding complexes of H_3Ssucc is always one less. [b] Average difference in the calculated and experimental titration curves expressed in cm^3 of the titrant.

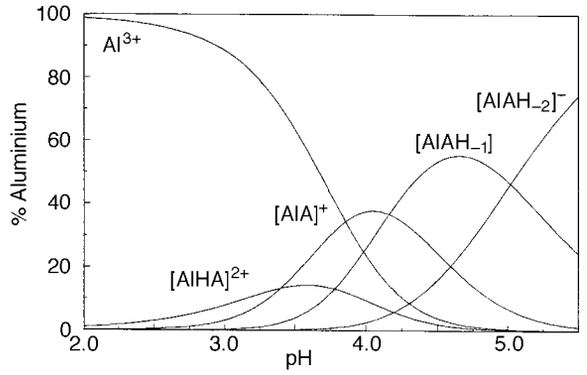


Fig. 5 Species distribution curves in the Al^{III}–H_2succ system; $c_{succ} = 0.015$, $c_{Al} = 0.003$ mol dm^{-3}

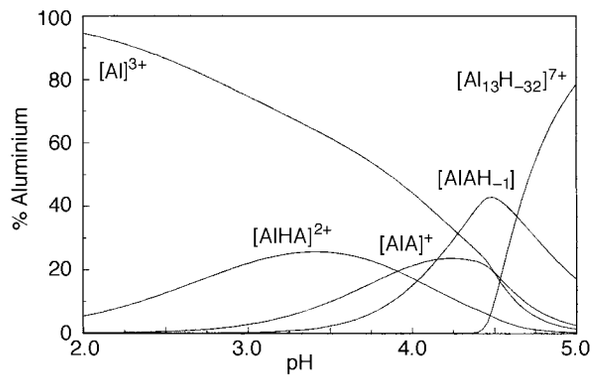


Fig. 6 Species distribution curves in the Al^{III}–Ac-Asp system; $c_{Ac\text{-}Asp} = 0.015$, $c_{Al} = 0.003$ mol dm^{-3}

likely, similarly to alcoholic OH and amide NH groups which exhibited an enhanced metal-binding ability when they were in a central position within the molecule.[19,20] To obtain information about the binding ability of the NH_2 group in Asp, its *N*-acetyl derivative (with a blocked amino group) and succinic acid (without the central amino group) were also studied. An attempt was first made to evaluate the pH-metric titration data on Al^{III}–Glu and –Asp systems with the same speciation model as accepted for simple amino acids, taking into account that the ligand contains an extra proton on the terminal carboxylic function. For Glu a reasonably good fit was obtained with the species and stability constants listed in Table 3. However, the Al^{III}–Asp system gave a very poor fit with this model, especially in the high-pH range (the Al^{III}–Asp system could be measured up to pH ≈6 without precipitation or unstable pH-meter readings, while in the Al^{III}–Glu system precipitation occurred at pH ≈5). When another deprotonated complex, $[AlAH_{-2}]$, was also included in the model a reasonable fit was obtained between the experimental and calculated titration data. The aluminium(III) systems of the two reference compounds *N*-Ac-Asp and H_2succ were fitted with the same speciation model as for Asp, with the exception that in the case of the Ac-Asp system the pH range evaluated was about one unit narrower due to precipitation. The results of the computer evaluation of the pH-metric data are shown in Table 3. Species distribution diagrams for the Al^{III}–H_2succ, –Ac-Asp and –Asp systems are depicted in Figs. 5–7.

The complex $[Al(HA)]^{2+}$ found for H_2succ and Ac-Asp is a monodentate carboxylate-co-ordinated species; its formation constant $\{Al^{3+} + AH^- \rightleftharpoons [Al(HA)]^{2+}\}$ is in reasonably good agreement with those obtained by Öhman[16] for simple aliphatic and aromatic carboxylates. With these ligands, unlike simple amino acids, the absence of the positively charged NH_3^+ group will not hinder the formation of such monocarboxylate-co-ordinated species. The consecutive loss of two protons from this $[Al(HA)]^{2+}$ species can be ascribed to the

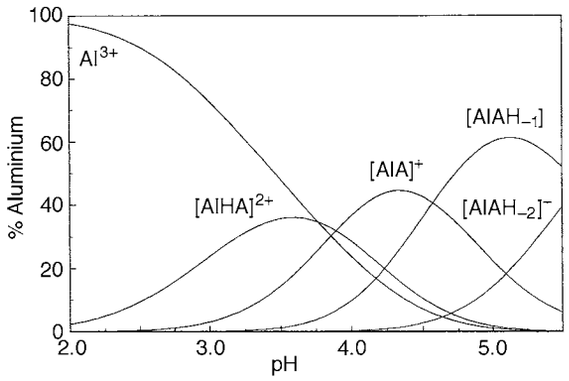
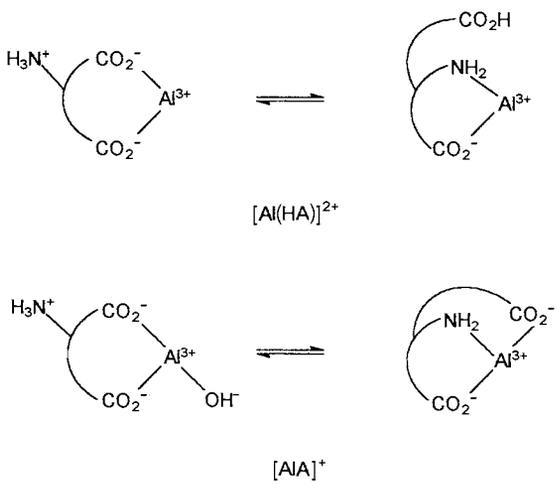


Fig. 7 Species distribution curves in the Al^{III}–Asp system; $c_{Asp} = 0.015$, $c_{Al} = 0.003$ mol dm^{-3}



Scheme 1

formation of a seven-membered (CO_2^-, CO_2^-) chelate and the ionization of a co-ordinated water molecule. The basicity-adjusted stability constants, which take into account the differences in basicity of the co-ordinating donor groups, are practically the same for Ac-Asp and H_2succ, confirming the same binding modes in their corresponding complexes. The presence of the bulky *N*-acetyl moiety in Ac-Asp, however, will result in the early formation of a precipitate, which can be either $Al(OH)_3$ or perhaps the neutral $[AlAH_{-1}]$ complex, from an oversaturated solution.

When the Al^{III}-binding properties of H_2succ and Ac-Asp are compared with those of Asp it can be seen that Asp does not form monodentate CO_2^--co-ordinated species (its stoichiometry should be $[AlAH_2]^{3+}$), because of the electrostatic repulsive effect of the protonated NH_3^+ group. However, when the stability constants of the (CO_2^-, CO_2^-) chelates are compared Asp displays significantly higher stability. In the case of Asp this species has a stoichiometry of $[Al(HA)]^{2+}$, due to the protonated NH_3^+ group. Assuming only bidentate (CO_2^-, CO_2^-) co-ordination in the $[Al(HA)]^{2+}$ (with protonated NH_3^+) and $[AlA]^+$ (mixed-ligand hydroxo species with protonated NH_3^+) complexes of Asp, the corresponding basicity-adjusted stability (proton-displacement) constants are about two orders of magnitude larger for Asp (see the last two rows of Table 3), which strongly suggests the involvement of the NH_2 group in binding to Al^{III}. Hence, we can assume two microforms for both Asp complexes, $[Al(HA)]^{2+}$ and $[AlA]^+$ (see Scheme 1). A similar tridentate (CO_2^-, NH_2, CO_2^-) co-ordination of iminodiacetate to Al^{III} was demonstrated by multinuclear NMR[21,22] and X-ray analysis in the solid state.[22] It is worthy of mention that even the corresponding Glu complexes exhibit an enhanced stability, and thus NH_2 involvement *via* the formation of the microform with a five- + seven-membered joint chelate

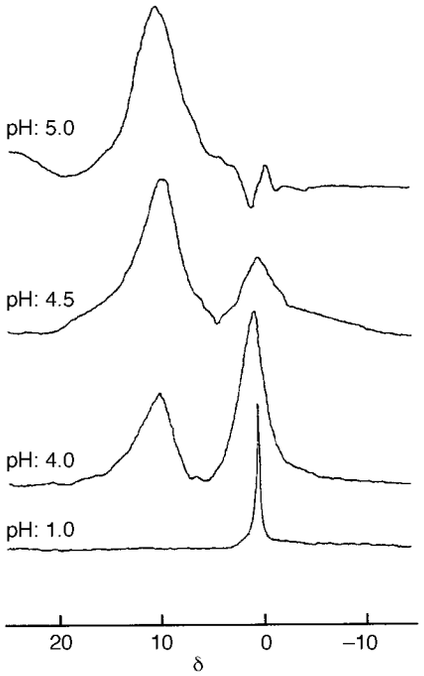


Fig. 8 Aluminium-27 NMR spectra of Al^{III}–Asp at 1:5 metal to amino acid ratio at different pH values; $c_{Al} = 0.005$ mol dm^{-3}

system can also be assumed. The formation of the complex $[AlAH_{-2}]^-$ likewise suggests co-ordination of the amino group, as this stoichiometry can be ascribed to only one reasonable binding mode: (CO_2^-, NH_2, CO_2^-) tridentate co-ordination of the ligand and two OH^- groups around the metal ion. Mixed hydroxo complexes of Al^{III} with three OH^- groups have never been detected with any ligand.

Aluminium-27 NMR measurements. The multinuclear NMR measurements point to the above discussed binding properties of the ligands. In contrast with simple amino acids, the ^{27}Al NMR spectra indicate a much stronger interaction (see Fig. 8) with Asp. The relatively sharp signal ($\Delta\nu \approx 130$–180 Hz) at around δ 10 suggests octahedral Al^{III} in a fairly symmetrical chemical environment in the Al^{III}–Asp complex formed at pH >4. Succinic acid reveals somewhat similar ^{27}Al NMR; behaviour. At pH 4.2 it displayed two peaks, at δ 0.5 and 4.6; the former is due to free Al^{3+}, while the latter, broader resonance is attributed to the $[AlA]^+$ with (CO_2^-, CO_2^-) chelation. From a study of the ^{27}Al NMR spectra of a series of Al^{III}–aminopolycarboxylate complexes, Karweer *et al.*[21] demonstrated a nearly linear relationship between the chemical shift and the denticity of the ligand. They established that replacement of a H_2O molecule by a nitrogen or oxygen donor with the formation of five-membered chelate rings produces an average deshielding of 6–7 ppm, while replacement of H_2O by OH^- in octahedral geometry results in deshielding by ≈2 ppm. Although no data have been reported for the deshielding effect of the formation of six- or seven-membered chelates, it is reasonable to assume that it decreases with increasing ring size (weaker interaction). Accordingly, the resonance at δ 4.6 for seven-membered (CO_2^-, CO_2^-) co-ordination (Al^{III}–H_2succ system) and that at δ 10.0 for five- +six-membered (CO_2^-, NH_2, CO_2^-) chelation (Al^{III}–Asp system) conform with the relationship reported by Karweer *et al.*[21]

2-Sulfanylsuccinic acid, which is a thiol analogue of Asp where the NH_2 group is replaced by an SH group, displays similarity to H_2succ in its Al^{III}-binding properties. The proton-displacement constants listed in the last two rows of Table 3 indicate a slightly enhanced stability of the complexes $[AlA]$ and $[AlAH_{-1}]^-$ as compared with these of H_2succ. However, this is even less than was found for Al^{III}–Glu complexes; hence, we assume that (CO_2^-, CO_2^-) chelation is the basic bonding mode in the Al^{III}–H_3S succ complexes.

Conclusion

In accordance with earlier reports, simple α-amino acids proved to be weak binders of Al^{3+}. However, their complexation becomes distinguishable from hydrolysis of Al^{3+} at high aluminium concentrations (0.5 mol dm^{-3} at a 1:1 metal ion to amino acid ratio) or at mmol dm^{-3} Al^{3+} with amino acid concentrations greater than 20 mmol dm^{-3}, and it could be detected unambiguously by means of both pH-potentiometric and ^1H, ^{13}C and ^{27}Al NMR measurements. With the tridentate Asp, which contains two carboxylate-O and one central amino-N binding donors, a much stronger interaction was observed. No such strong complexation could be detected with either H_2succ or Ac-Asp, which lack the central amino binding site. These results strongly suggest that, besides the negatively charged CO_2^- donors, in the event of a favourable steric arrangement, the NH_2 group (not only in amino acids, but probably also in peptides and proteins) can likewise participate in the binding of Al^{III}. The binding ability of thiolate S^-, however, could not be verified in H_3S succ, although it occurs in a similarly favourable steric environment.

Acknowledgements

This work was supported by the Hungarian Academy of Sciences (Project No. OTKA T7458/1993 and T14075/1995).

References

1 E. Marklund and L. O. Öhman, *Acta Chem. Scand.*, 1990, **44**, 353.
2 S. Singh, S. Gupta, P. C. Yadava, R. K. P. Singh and K. L. Yadava, *Z. Phys. Chem. Leipzig*, 1986, **267**, 902.
3 R. B. Martin, in *Aluminium in Chemistry, Biology and Medicine*, eds. M. Nicolini, P. F. Zatta and B. Corain, Cortina International, Verona, 1991.
4 S. Dayde, Ph.D. Thesis, Université de Toulouse, 1990.
5 K. S. J. Rao and G. V. Rao, *Mol. Cell. Biochem.*, 1994, **137**, 61.
6 Ph. Charlet, J. P. Deloume, G. Duc and G. T. David, *Bull. Soc. Chim. Fr.*, 1984, I-222.
7 G. Gran, *Acta Chem. Scand.*, 1950, **29**, 559.
8 H. Irving, M. G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475 .
9 L. Zékány and I. Nagypál, in *Computational Methods for the Determination of Stability Constants*, ed. D. Legett, Plenum, New York, 1985.
10 L. O. Öhman and S. Sjöberg, *Acta Chem. Scand., Sect. A*, 1982, **36**, 47.
11 R. G. Bates, *Determination of pH*, Wiley, New York, 1964.
12 I. Fábián and I. Nagypál, *Talanta*, 1982, **29**, 71.
13 C. Orvig, in *The Coordination Chemistry of Aluminum*, ed. G. H. Robinson, VCH, New York, 1993.
14 J. M. Garrison and A. L. Crumbliss, *Inorg. Chem.*, 1987, **26**, 3660.
15 T. Kiss, I. Sóvágó and R. B. Martin, *Inorg. Chem.*, 1991, **30**, 2130.
16 L. O. Öhman, *Acta Chem. Scand.*, 1991, **45**, 258.
17 L. O. Öhman and S. Sjöberg, *J. Chem. Soc., Dalton Trans.*, 1983, 2513.
18 T. Kiss, in *Biocoordination Chemistry*, ed. K. Burger, Ellis Horwood, New York, 1990.
19 T. Kiss, Cs. Simon and Zs. Vachter, *J. Coord. Chem.*, 1987, **16**, 225.
20 I. Sóvágó, in *Biocoordination Chemistry*, ed. K. Burger, Ellis Horwood, New York, 1990.
21 S. B. Karweer, B. P. Pillai and R. K. Iyer, *Magn. Reson. Chem.*, 1990, **28**, 922.
22 S. P. Petrosjanc, M. A. Maljarik and A. B. Iljuhin, *Zh. Neorg. Khim.*, 1995, **40**, 769.

Received 6*th December* 1996; *Paper* 6/08250E