Definitive identification of lead(ii)-amino acid adducts and the solid state structure of a lead–valine complex†

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Electrospray ionization mass spectra of lead(ii) nitrate–amino acid mixtures enable unequivocal identification of lead complexes for each of the essential amino acids and a valine complex is reported as the first crystallographically characterized lead–amino acid complex.

The toxic effects of lead compounds are manifested in anemia, neuropathy and hypertension.[1–3] Inhibition of heme synthesis is established as a principal biochemical action[4] and a key lead-enzyme interaction has been modeled.[5] Crystallographic studies of some proteins are augmented using Pb(iv) and Pb(ii) labels,[1] and one may expect amino acids to coordinate with most metals and metalloids. However, known amino acids complexes of lead are incompletely characterised.[1,6]

We have used electrospray ionization mass spectrometry (ESI-MS) to unequivocally identify complexes of lead with all of the naturally occurring amino acids. The results contrast the limited number of metal-amino acid adducts observed for As(iii), Sb(iii) and Bi(iii)[7] under similar experimental conditions. Moreover, the data complement the recent observations of lead-glucose cations by ESI-MS.[8,9]

ESI-MS data for 50% ethanolic solutions containing $Pb(NO_3)_2$ and a stoichiometric equivalent of an amino acid‡ are summarised in Table 1. Prominent *m/z* peaks (highest peak amongst the cluster of isotopic peaks) are listed together with their relative intensity and monocationic assignment (given as a Pb : Am ratio, Am = amino acid), all of which have been supported by MS/MS experiments, but are recognizable by virtue of the distinctive isotopic array [^{204}Pb (1.48 %), ^{206}Pb (23.6 %), ^{207}Pb (22.6 %), ^{208}Pb (52.3 %)] naturally exhibited by lead. Most reaction mixtures exhibit cations with 1 : 1, 1 : 2, 2 : 2 and 2 : 3 Pb : Am ratios. L-Threonine, L-methionine and L-aspartic acid also adopt a 3 : 2 Pb : Am ratio, and a representative spectrum of the L-threonine mixture shown in Fig. 1 illustrates the peaks assigned to a complex of 3 : 3 Pb : Am ratio. Cations (not listed in Table 1) of lead with alanine, valine, histidine, glutamic acid, arginine or proline are observed to contain nitrate groups and/or water molecules and L-arginine engages lead with 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5 and 1 : 6 Pb : Am ratios. Although lead is known to inhibit coordination of cysteine residues to zinc[10] and adopt thiolate complexation,[11] our ESI-MS data does not implicate a preference for complexation of cysteine over other amino acids, as observed for bismuth.[7]

ESI-MS provides insight into the coordination chemistry of metals in the gas phase, but cannot be directly correlated to species in solution or in the solid state. Nevertheless, it is informative to consider specific structural features of metal-amino acid interactions. To this end, we have isolated crystalline material§ that has been confirmed as the valine complex shown in Fig. 2, representing the first crystallographically characterised lead-amino acid complex.¶

An eight-coordinate holodirected[12,13] environment is observed for lead, imposed by oxygen centers through chelation from one valine ligand, a second monodentate valine ligand, two water
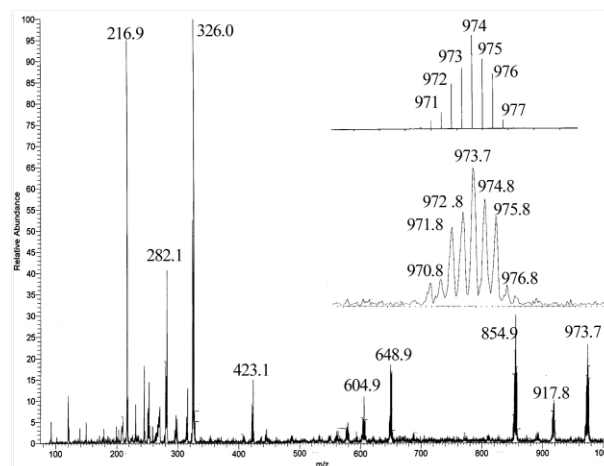
Fig. 1 ESI-MS of lead(ii) nitrate and L-threonine in 50 % ethanol–water. Peak assignments of lead and amino acid containing monocations can be found as Pb:Am ratios in Table 1. The calculated (upper expansion) isotopic array and observed (lower expansion) isotopic array for a complex (max. int. 974) consistent with a 3 : 3 Pb : threonine ratio is shown.
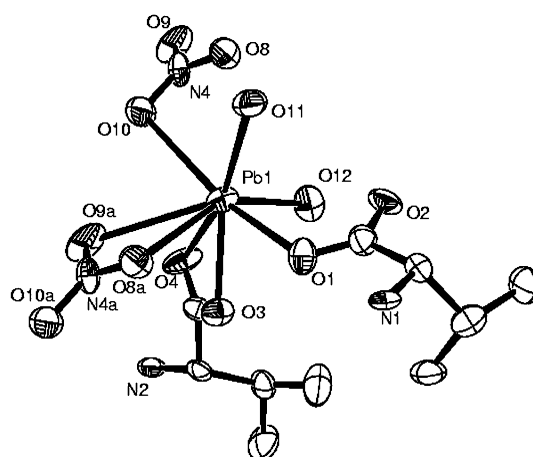

Fig. 2 Crystallographic view of the coordination environment of lead in $Pb(Val)_2(H_2O)_2(NO_3)_2$. Thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å): Pb1–O3 2.36(1), Pb1–O1 2.44(1), Pb1–O12 2.50(1), Pb1–O8a 2.78(1), Pb1–O9a 2.85(2), Pb1–O4 2.87(1), Pb1–O11 2.89(1), Pb1–O10 2.95(1).

molecules, interaction from one nitrate group (O10) and chelation from a symmetry related nitrate group (O8 and O9) of the next complex resulting in a polymeric solid state structure. A second nitrate ion that does not interact with lead is not shown in Figure 2. The charge balance for lead(ii) requires that the pendant nitrogen centers of the valine ligands represent ammonium groups, so that the complex is best considered as two neutral valine ligands on $Pb(NO_3)_2$. Although, dications are not apparent in any of the spectra, an *m/z* peak observed at 441 in the ESI-MS of the lead nitrate–valine mixture is assigned to the monocation $[Pb(Val)_2]^+$ (Table 1).

Table 1 ESI-MS data for lead(II) nitrate–amino acid reaction mixtures in 50% ethanol–water for spectra that show prominent mono-cation peaks (*m/z*) containing lead and amino acid units. All MS assignments have been supported by MS/MS, and are listed as lead : amino acid ratios or fragment ions

	m/z	Assignment Pb:Am	% (Rel. int.)		*m/z*	Assignment Pb:Am	% (Rel. int.)
Gly	282.1	1 : 1	100	**Cys**	328.1	1 : 1	100
	636.9	2 : 3	35		448.9	1 : 2	55
Ala	296.1	1 : 1	90		654.9	2 : 2	50
	385.0	1 : 2	25	**HCys**	342.1	1 : 1	100
	677.9	2 : 3	100		474.8	1 : 2	15
Val	324.0	1 : 1	100		680.9	2 : 2	55
	441.0	1 : 2	45	**Asp**	340.1	1 : 1	100
	761.9	2 : 3	75		677.0	2 : 2	55
Leu	338.0	1 : 1	10		882.9	3 : 2	10
	469.0	1 : 2	35		1015.9	3 : 3	30
	673.1	2 : 2	15	**Glu**	354.0	1 : 1	100
	804.0	2 : 3	100		558.1	2 : 1	10
Ile	338.0	1 : 1	10		705.0	2 : 2	30
	469.0	1 : 2	35	**Asn**	339.0	1 : 1	100
	673.1	2 : 2	15		470.9	1 : 2	12
	804.0	2 : 3	100		676.9	2 : 2	20
Phe	372.1	1 : 1	85		806.9	2 : 3	50
	534.9	1 : 2	25	**Gln**	353.1	1 : 1	80
	740.8	2 : 2	15		667.0	$C_{10}H_{13}N_4O_4Pb_2^+$	50
	905.9	2 : 3	100		684.8	$C_{10}H_{15}N_4O_5Pb_2^+$	75
Trp	411.1	1 : 1	100		703.0	2 : 2	100
	614.9	1 : 2	30		848.9	2 : 3	74
	818.9	2 : 2	5	**Lys**	335.3	$C_5H_7N_2O_2Pb^+$	50
	1023.1	2 : 3	35		353.1	1 : 1	40
Tyr	388.1	1 : 1	100		460.8	$C_{12}H_{23}N_4O_2Pb^+$	100
His	362.1	1 : 1	100		850.0	2 : 3	80
	723.0	2 : 2	25	**Arg**	381.0	1 : 1	50
	875.8	2 : 3	10		555.1	1 : 2	100
Ser	312.1	1 : 1	95		728.9	1 : 3	40
Thr	326.0	1 : 1	100		902.9	1 : 4	20
	648.9	2 : 2	40	**Pro**	322.0	1 : 1	35
	854.9	3 : 2	39		437.1	1 : 2	99
	973.7	3 : 3	30		758.0	2 : 3	100
Met	356.1	1 : 1	100				
	504.9	1 : 2	7				
	709.1	2 : 2	5				
	857.9	3 : 2	50				

We have demonstrated the ESI-MS technique as a data-rich approach to the identification of lead complexes involving amino acids. We envisage a diverse utility of the technique to study interactions of other small and larger biomolecules with all heavy metals.

methods using SHELXS86 (Sheldrick, G.M., 1985) and refinement was done using full matrix least squares on F^2 using 3320 independent reflections with $I > 3.00\sigma(I)$. CCDC 216995. See http://www.rsc.org/suppdata/cc/b3/b311599b/ for crystallographic data in .cif or other electronic format.

References and Notes

‡ An equimolar (1 mmol) mixture of Pb(NO$_3$)$_2$ and the amino acid was stirred overnight in 10 mL distilled water and 10 mL ethanol at RT and then suction filtered. Samples were injected directly at a flow rate of 1.2 mL min^{-1} into the electrospray source of a Finnigan LCQ Duo ion trap mass spectrometer with a spray voltage of 4.0 kV and capillary temperature of 200 °C. MS/MS spectra were obtained using helium as a collision gas and an optimised collision energy.

§ The filtrate from the Pb(NO$_3$)$_2$, L-valine solution described above was suction filtered and left to evaporate for 1 week giving white needles of Pb(Val)$_2$(H$_2$O)$_2$(NO$_3$)$_2$, yield: 0.59 mmol (55 %), mp: 105 °C; FT-IR (cm^{-1}): 1515(1), 1568(2), 1613(3), 1632(4) 1768(5), 1022(6), 804(7), 752(8), 586(9). 558(10).

¶ Crystal data for Pb(Val)$_2$(H$_2$O)$_2$(NO$_3$)$_2$: C$_{10}$H$_{26}$N$_4$O$_{12}$Pb, $M = 601.53$, orthorhombic, space group $P2_12_12_1$ (no. 19), white needles, $a = 13.609(6)$ Å, $b = 26.75(1)$ Å, $c = 5.416(5)$ Å, $V = 1971(2)$ Å^3, $D_c = 2.027$ Mg m^{-3}, $Z = 4$, $T = 173$ K, $R = 0.0427$ ($F^2 > 2\sigma$), w$R2 = 0.1092$ (all data), Flack parameter = 0.03(3), GOF = 0.966. The structure was solved by direct

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