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Dimethyltin(IV) cation induced amide deprotonation of aspartic acid containing dipeptides †

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The co-ordination behaviours of two dipeptides, glycyl-aspartic acid (Gly-Asp) and aspartyl-glycine (Asp-Gly), towards dimethyltin(IV) cations have been investigated by potentiometry and spectroscopic methods (^{1}H and ^{13}C NMR and Mössbauer). The formation of mononuclear complexes has been detected between pH 2 and 10, although the hydrolysed species are also present in the solution. On the basis of pH-metric and NMR data, the carboxylate groups are bound to the metal ion in the acidic pH range and act as anchoring groups for the metal-promoted deprotonation of amide nitrogens between pH 4 and 7. The complexes formed in this way are relatively inert on the NMR timescale in the case of both ligands, allowing their structural characterization by NMR spectroscopy. These species can be described with a trigonal bipyramidal structure having a $\{CO_2^-, N^-_{amide}, NH_2\}$ co-ordination, where the metal-bound carboxylate is very likely the C-terminal one. In spite of the constitutional differences, Asp-Gly and Gly-Asp form stable complexes with dimethyltin(IV) cation in the neutral pH range with identical structure and geometry.

Introduction

The increasing interest in the chemistry of organotin compounds has led to extended studies on their interactions with different biomolecules *e.g.* carbohydrates,[1–3] nucleic acid derivatives,[4–6] amino acids[7–10] and peptides.[11–15] Several reports revealed the versatile co-ordination chemical behaviour of organotin cations toward molecules containing different types of donor sets *e.g.* {O},[1–7] {O,N}[7,9,10,15] or {S,O,N}[9] including both solid state and solution studies. The industrial, agricultural and biological applications of organotin(IV) compounds increased dramatically between 1960 and 1985 (40000 t in 1985).[16] Although the consumption of organotin(IV) chemicals has fallen in recent years, the above mentioned uses result in their continuous accumulation in the environment. These compounds are generally very toxic, even at low concentration. On the other hand, many dialkyltin derivatives have been found to possess anticancer effects on different tumour cells and their structures in the solid state are well characterized.[17–19] In spite of these efforts, the mechanisms of action of these drugs in the living cell are still unsolved. Their interaction with nucleic acids and peptides might play a special role in their biological activity. Evidence for the co-ordination of dimethyltin(IV) cation to phosphate groups of DNA or DNA fragments has been found both in solution[4] and in the solid state[20] but their after-effect is unclear. Di- or tri-alkyltin cations may also bind to proteins and glycoproteins of cell membranes as well as to cellular proteins, *e.g.* $Bu^t_2Sn^{2+}$ or $Bu^t_3Sn^+$ to ATPase and acetylcholine esterase of human erythrocyte membrane[21] or Et_3Sn^{2+} to ATPase and hexokinase of trout, feline and human erythrocytes.[22] Modelling the interaction between organotin cations and proteins or peptides by low molecular weight mimics in aqueous solution may be fruitful for the better understanding of these interactions. Solid state studies on the interaction of dialkyltin(IV) cations with several peptides revealed trigonal bipyramidal complexes with terminal amino, deprotonated amide and carboxylate co-ordination.[11,12,23] In a previous study we reported the co-ordination behaviour of dimethyltin(IV) cation toward Gly-Gly and Gly-His and some related ligands containing mainly imidazole ring and carboxylate as donor groups.[15] Only the above mentioned two dipeptides were found to co-ordinate to the dimethyltin(IV) cation strongly enough to suppress its hydrolysis. Our results provided the first example that dialkyltin(IV) cations are able to promote peptide nitrogen deprotonation in aqueous solution too, at unexpectedly low pH. Metal binding to the imidazole ring, which was suggested as a binding site for alkyltin(IV) cations in several proteins,[24] was not observed under the conditions used.

In this paper we report the continuation of our systematic investigations on the interaction of dipeptides with dimethyltin(IV), in order to clarify the alkyltin–protein interaction by varying the side-chain donor group. O-Donor ligands, such as carboxylates, interact strongly with organotin(IV) cations,[8] especially when chelate co-ordination may occur. Dimethyltin(IV) complexes of two dipeptides (glycylaspartic acid (Gly-Asp) and aspartylglycine (Asp-Gly)), containing carboxylate groups as side-chain donors, have been investigated in aqueous solution by potentiometry, ^{1}H and ^{13}C NMR and Mössbauer spectroscopic methods. The binding mode of the ligands, the structure and geometry of the complexes formed in solution are also discussed.

Experimental

Materials

Glycylaspartic acid and aspartylglycine (Aldrich) and dimethyltin(IV) dichloride (Fluka) were used without further purifi-

† Electronic supplementary information (ESI) available: ^{1}H–^{1}H COSY and ^{1}H NMR spectra. See http://www.rsc.org/suppdata/dt/a9/a909741d/

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cation. A fresh dimethyltin(IV) dichloride solution was prepared and standardized by acid–base titration every 2 days. pH-Metric titrations were performed using NaOH (Fluka) standard solution.

pH-Metric measurements

The protonation and co-ordination equilibria were investigated by potentiometric titration in aqueous solution ($I = 0.1$ mol dm^{-3}, $NaClO_4$, and $T = 298 \pm 0.1$ K) using an automatic titration set including a Dosimat 665 (Metrohm) autoburette, an Orion 710A precision digital pH-meter and an IBM-compatible PC. The Orion 8103BN semimicro pH glass electrode was calibrated[25] using the modified Nernst equation (1)

$$E = E_0 + K\cdot\log[H^+] + J_H[H^+] + J_{OH}K_w[H^+]^{-1} \quad (1)$$

where J_H and J_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction and to the alkaline and acidic errors of the glass electrode; $K_W = 10^{-13.75}$ mol^2 dm^{-6} is the autoprotolysis constant of water.[26] The parameters were calculated by a non-linear least squares method. The species formed in the systems were characterized by the general equilibrium process (2)

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \quad (2)$$

where M denotes the dimethyltin(IV) cation and L the non-protonated ligand molecule. Charges are omitted for simplicity, but can easily be calculated since the composition of the fully protonated dipeptides is described as H_3L^+. The formation constants were calculated by means of the computer program PSEQUAD.[27]

The protonation and complex formation constants were determined from 4 and 7 independent titrations (80–110 data points per titration), respectively. The metal-to-ligand ratios varied between 1:1 and 1:3, with the dimethyltin(IV) concentration between 1×10^{-3} and 4×10^{-3} mol dm^{-3}. The pH-metric data between pH 1.8 and 11.2 were used for the evaluation.

NMR measurements

^{1}H and ^{13}C NMR measurements were performed at 400 and 100.6 MHz, respectively, on a Bruker DRX400 spectrometer. The chemical shifts δ were measured with respect to 1,4-dioxane as an internal reference and converted relative to TMS, using $\delta_{dioxane}$ 3.70 for ^{1}H and 67.4 for ^{13}C NMR. ^{13}C peak assignments for the bound molecules were made by ^{1}H undecoupled experiments and by 2-D ^{13}C–^{1}H correlation experiments. The individual chemical shifts and ^{1}H–^{117,119}Sn coupling constants of the different hydrolysed species of the dimethyltin cation, as well as the geometry of the complexes, were reported in detail earlier.[4,15] The individual $^2J(^{119}Sn–^1H)$ and $^1J(^{119}Sn–^{13}C)$ heteronuclear couplings can be "converted" into C–Sn–C angles by using the published equations.[28,29]

For ^{1}H NMR measurements the ligand concentration was 0.01 mol dm^{-3} for both dipeptides and the metal concentration 0.005 mol dm^{-3} (in some cases, spectra were also performed with [L] = [M] = 0.01 mol dm^{-3}). In the case of ^{13}C NMR the concentrations used for the ligand and metal were 0.15 and 0.075 mol dm^{-3}, respectively. ^{1}H NMR spectra were also recorded at the latter concentrations at neutral pH. Measurements were generally made in a 9:1 H_2O:D_2O mixture. In a few cases they were performed in pure D_2O.

Mössbauer measurements

The ^{119}Sn Mössbauer spectra of quick-frozen solutions were recorded on a Ranger spectrometer in a constant acceleration mode, using a source ($BaSnO_3$) with an activity of 0.3 GBq. For the determination of isomer shift (IS) and quadrupole splitting (QS) values computer evaluation was used. The reproducibility of the Mössbauer parameters was ±0.02 mm s^{-1} for IS and ±0.04 mm s^{-1} for QS values in each measurement. The IS values are referred to that of $BaSnO_3$. The experimental quadrupole splitting (QS_{exp}) values were compared with the calculated ones, assuming different stereochemistries of the co-ordination sphere of tin(IV), according to the point charge model formalism.[30,31]

Table 1 Formation constants and derived data for the dimethyltin(IV) complexes of Gly-Asp and Asp-Gly (as their logarithms) at $T = 298$ K, $I = 0.1$ M $NaClO_4$; $\beta_{pqr} = M_pL_qH_r/[M]^p[L]^q[H]^r$ with estimated errors in parentheses (last digit). The formation constants of the hydrolytic species are as follows: log $\beta_{10\text{-}1} = -3.175(5)$, log $\beta_{10\text{-}2} = -8.415(4)$, log $\beta_{10\text{-}3} = -19.459(4)$, log $\beta_{20\text{-}2} = -4.95(4)$, log $\beta_{20\text{-}3} = -9.96(3)$

pqr	Gly-Asp	Asp-Gly
011	8.36(1)	7.99(1)
012	12.64(1)	11.58(1)
013	15.38(2)	14.30(1)
112	14.5(2)	13.4(1)
111	11.6(1)	10.4(1)
110	7.51(8)	6.90(5)
11 − 1	2.30(5)	2.13(2)
log K^a	1.89	1.83
pK(MH_2L)	2.97	3.02
pK(MHL)	4.05	3.49
pK(ML)	5.21	4.77
ΔpK^b	1.31	0.56
Experimental points	649	665
Fitting parameter (mL)	0.007	0.004

^{a} log K = log β_{112} − log β_{012} refers to the stability constants of the monodentate, carboxylate co-ordinated complexes. ^{b} $\Delta pK = pK_2 - pK(MH_2L)$ (pK_2 = log β_{012} − log β_{011}).

Results and discussion

Hydroxo-complexes of dimethyltin(IV)

Dimethyltin(IV) cation forms stable and soluble mono- and di-nuclear hydroxo-complexes in the pH range studied. The formation constants of these species, determined earlier,[15] were taken into consideration during the evaluation of the pH-metric data.

Complexes of the studied ligands

The protonation and formation constants of the species formed in the two systems, together with some calculated data, are collected in Table 1. The pK values of the ligands agree well with the earlier reports.[32,33] Some representative species distribution curves, calculated for the concentrations of the pH-metric and ^{1}H NMR measurements, are also presented on Fig. 1. As concerns the composition of the species formed, the two systems are very similar. Although the peptide–dimethyltin(IV) complexes dominate at slightly acidic and neutral pH, hydrolytic species are also present in the whole pH range. The calculated stability constants of the complex MH_2L (1.89 for Gly-Asp and 1.83 for Asp-Gly) are typical for complexes with monodentate carboxylate co-ordination. The formation of MH_2L causes only slight shifts in the ^{1}H NMR spectra between pH 1.8 and 3.8, due to the weak and labile interaction of the dipeptides and dimethyltin(IV) cation. The deprotonation of these species take place with p$K \approx 3$ (shown in Table 1) and could be assigned to three different processes: deprotonation of the second carboxylate group, a metal-bound water molecule or the amino group. The last two possibilities can be rejected due to the following reasons. The co-ordination of a negatively charged group (*e.g.* a carboxylate group) to the dimethyltin(IV) ion should increase the pK of a co-ordinated water molecule,[10,15] by weakening of the dimethyltin(IV)–water

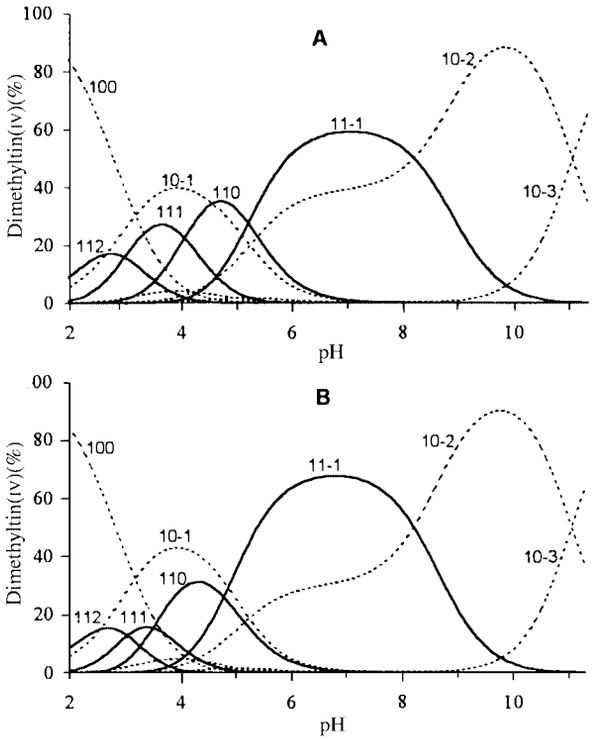


Fig. 1 Species distribution curves in the dimethyltin(IV)–Gly-Asp (A) and –Asp-Gly (B) systems. Hydrolytic species are shown by dotted lines. For clarity, curves belonging to the $M_2(OH)_2$ and $M_2(OH)_3$ complexes (minor species between pH 3 and 5) are not labelled ([M] = 0.005 mol dm^{-3}, [L] = 0.01 mol dm^{-3}). The notation of the different species corresponds to the *pqr* values of the corresponding complex $M_pL_qH_r$.

bond. The observed p*K*s, however, are *ca*. 0.2 log unit lower than log β_{10-1} (−3.17). The approximately 6 log units decrease of the amino p*K*s compared to those of the "free" ligands would only be explained by a strong extra stabilization of the complex MHL *e.g*. formation of a stable 5–6 membered chelate ring as in the case of amino acids.[8] A 6-membered chelate ring could form in the case of Asp-Gly with the participation of the side chain carboxylate group but such stable chelate formation cannot happen with Gly-Asp. These facts are inconsistent with the observed low and almost equal p$K(MH_2L)$ values.

According to the above, the $MH_2L \longrightarrow MHL$ process can be assigned to the deprotonation and co-ordination of the second carboxylate group. Although the observed p$K(MH_2L)$ values are very similar, the decrease of the second p*K*, induced by the metal co-ordination, is rather different in case of the two dipeptides (Δp*K* = 1.31 for Gly-Asp and 0.56 for Asp-Gly, see Table 1). The simultaneous co-ordination of both carboxylates forms a 7-membered chelate ring in the complex MHL of Gly-Asp which is more favoured than either the macrochelate or the fused chelate rings (with the participation of the amide C=O group) in the same complex of Asp-Gly. The deprotonation of a dimethyltin(IV) bound water molecule takes place during the $MHL \longrightarrow ML + H^+$ process. Owing to the above structural differences, the p*K* of the complex MHL in the Gly-Asp containing system is more than 0.5 log unit higher as compared with the corresponding value of Asp-Gly (see Table 1). Below pH 5, where all the mentioned processes take place (Fig. 1), the ^{1}H NMR spectra show only slight differences between the "free" ligand and the metal containing systems. However, the pH-dependent ^{13}C NMR measurements indicated the formation of complexes having fast mutual exchange on the NMR timescale by an important (approximately 1 ppm) downfield shift of the signals of the carboxylate carbons in the presence of $(CH_3)_2Sn^{IV}$ as compared with the metal free system.

At higher pH further deprotonation was observed leading to species $MH_{-1}L$. These complexes are present in the solutions over a wide pH range (4.5–9.5) and are dominant around the neutral pH, even when the ligand concentration is relatively small (Fig. 1). Above pH 10 in both systems only the hydrolytic species of dimethyltin(IV) cation are present. The formation constants of the species ML with Gly-Gly,[15] Asp-Gly and Gly-Asp are 6.61, 6.90 and 7.51, respectively (the p*K* values of the above species are 4.81, 4.77 and 5.21). These values suggest higher extra stabilization due to the chelated co-ordination of the side chain carboxylate in the case of Gly-Asp, consistent with the mentioned structural differences. Again, the formation of $MH_{-1}L$ could be explained by different base consuming processes: (i) deprotonation of another metal bound water molecule (better described as $M(HL)(OH)_2$); (ii) proton release from the terminal amino group (ML(OH)); (iii) co-operative deprotonation of the terminal amino group and the peptide nitrogen, followed by the release of a water molecule from the co-ordination sphere ($MH_{-1}L$).

The latter mechanism was demonstrated earlier for two dipeptides in aqueous solution.[15] The presence of carboxylate groups as side chain donors in the investigated dipeptides, however, may change the co-ordination properties of the molecules, thus the attribution of these steps to the deprotonation of certain groups would be ambiguous without further spectroscopic information. ^{1}H and ^{13}C NMR spectroscopic measurements were found to be useful for the detailed investigation of these processes since the spectra showed significant changes, in parallel with the formation of the complex $MH_{-1}L$. New sets of signals appeared in the ^{1}H NMR spectra indicating a slow exchange between the complexes and the "free" ligands, relatively to the NMR timescale. The p*K* values calculated for the complex ML are smaller than (Asp-Gly) or similar to (Gly-Asp) the p*K* determined for the second hydrolysis step of the free cation ($pK_{(MOH)} = 5.24$). On the other hand, the presence of species in the solutions in slow mutual exchange cannot be explained by hydroxo mixed ligand complexes in which the ligands are bound to the dimethyltin(IV) cation *via* carboxylate groups only. This strongly suggests that metal-bound water deprotonation should be excluded from the possible pathways, leading to $MH_{-1}L$. The relative kinetic inertness of the species $MH_{-1}L$ also disfavours co-ordination of the amino group alone but detailed analysis of the measured NMR spectra is needed to determine the co-ordination mode of the ligands.

NMR information for the co-ordination mode of Gly-Asp

The assignments of the ^{1}H and ^{13}C NMR signals, together with the coupling constants observed, are given in Tables 2 and 3. The chemical shifts and the coupling patterns of the C*H*–CH_2 signals considerably change in presence of the metal ion (Table 2, Fig. 2A) as compared with the "free" ligand. Moreover, coupling can be detected between the tin nucleus and the CH hydrogen (^3J = 28.2 Hz). This may be considered as the effect of co-ordination of dimethyltin(IV) cation to the deprotonated amide nitrogen. Additionally, it can be noted that at pH 4.65 a coupling between the CH and the amide hydrogen is observed in case of the "free" ligand which disappears in the spectrum of the $MH_{-1}L$ complex, formed in *ca*. 10% at this pH.

Dramatic changes are observed also for the CH_2 group next to the amino group (Fig. 2B) above pH 4, when dimethyltin(IV) is present in the solution. The main feature is the presence of couplings with the NH_2 hydrogens, which shows that the slow exchange between the complex and the "free" ligand prevents fast exchange of the amino hydrogens with the solvent protons. This is a clear proof for the co-ordination of the amino group to the dimethyltin(IV) cation. It also has to be mentioned that neither CH_2 or NH_2 hydrogens are equivalent due to the binding of the amino group to the metal ion, which can be seen from the unsymmetrical shape of the CH_2 peaks of $MH_{-1}L$ (see Fig. 2B). (A similar splitting pattern was observed earlier for the same type of hydrogens in the ML and $MH_{-1}L$ complexes of

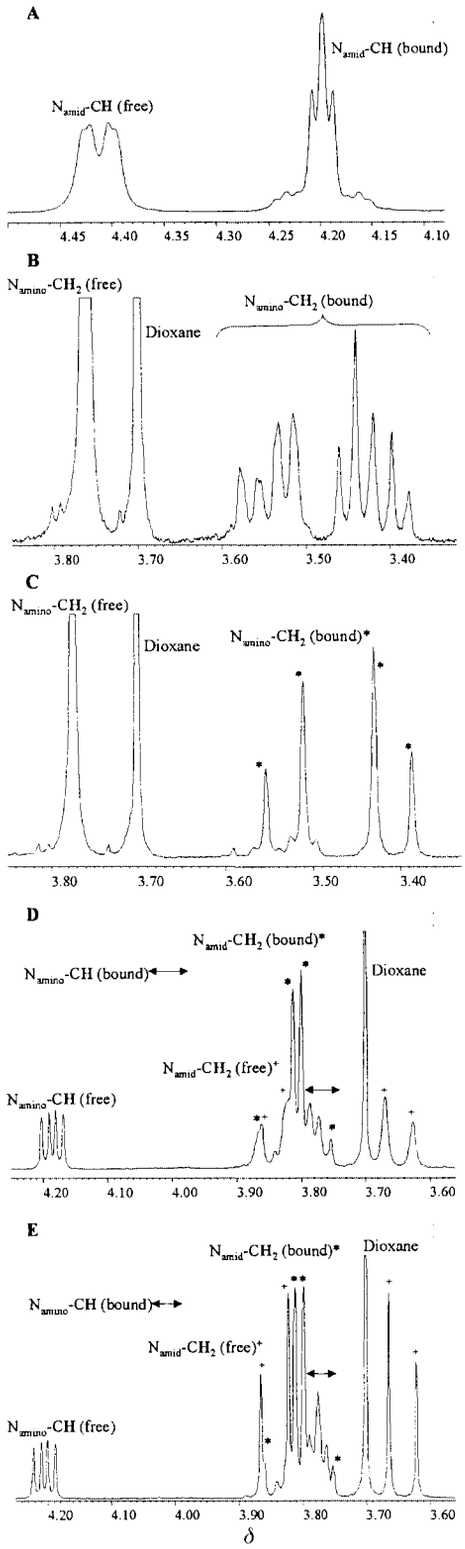


Fig. 2 Part of the ^{1}H NMR spectra of the dimethyltin(IV)–Gly-Asp (A–C) and –Asp-Gly (D, E) systems in water (A, B and D) or D_2O (C and E). [L] = 0.15 (A) or 0.01 mol dm^{-3} (B–E); [M] = 0.075 (A), 0.005 (B, C) or 0.01 mol dm^{-3} (D, E); pH 7.34 (A), 7.15 (B) or 6.82 (D); pD = 6.74 (C) or 6.47 (E).

Gly-His).[15] ^{1}H NMR spectra were also recorded in D_2O that provide further evidence for the co-ordination of the amino group (Fig. 2C). Indeed, the splitting of the CH_2 hydrogens with the amino protons disappears as a result of the $^1H \longrightarrow {}^2H$ exchange of the amino hydrogens. At the same time, coupling between one of the AB type CH_2 hydrogens and the $^{117/119}$Sn nuclei appears which cannot be detected in water due to the splitting of the signals (Fig. 2C, $^3J(^{117/119}Sn–^1H) = 11.7$ Hz).

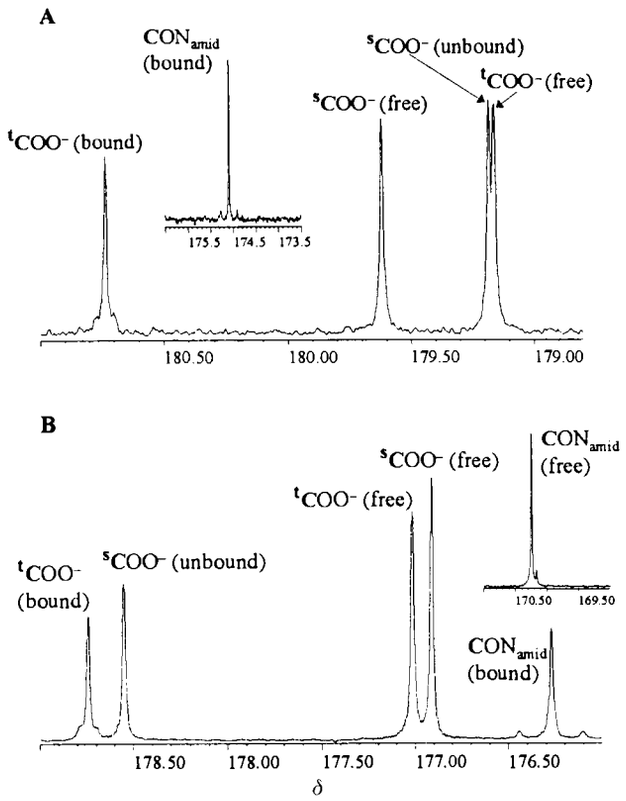


Fig. 3 Part of the ^{13}C NMR spectra of the dimethyltin(IV)–Gly-Asp (A) and –Asp-Gly (B) systems. (A) [L] = 2[M] = 0.15 mol dm^{-3}, pH 7.34; (B) [L] = 1.33[M] = 0.15 mol dm^{-3}, pH 5.10. Bound (A) and free (B) carbonyl signals are shown in the inserts, t and s superscripts denote the terminal and side chain carboxylate carbons, respectively.

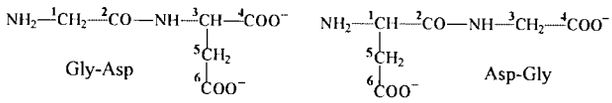
It is worth mentioning, that the difference between the chemical shifts of the magnetically inequivalent methyl hydrogens of the dimethyltin(IV) cation is 0.176 ppm and their two bond couplings with the ^{119}Sn nuclei also differ significantly, as is shown in Table 2.

The ^{13}C NMR measurements give further evidence for the co-ordination mode of Gly-Asp. The assignment of the ^{13}C peaks was carried out by means of simple ^{13}C 1-D measurements and a 2-D ^{13}C–^{1}H correlation experiment. The chemical shifts of the different carbons together with the observed coupling constants are presented in Table 3. Similarly to the ^{1}H NMR spectra, two sets of signals can be observed and the two methyl carbons of the dimethyltin(IV) cation in the complex $MH_{-1}L$ are inequivalent, as well. Among the two carboxylate groups of Gly-Asp only one shows coupling with tin (Fig. 3A), indicating its co-ordination to the metal ion. The assignment of the two carboxylate signals was made indirectly. pH dependent ^{13}C NMR titrations were performed both in the absence and in the presence of metal ion. The $CHCO_2^-$ carbon signals of the complex suffered only a slight shift with increasing pH, while the $CH_2CO_2^-$ signals shifted considerably between pH 4.5 and 6 as for the "free" ligand. This may suggest deprotonation of the side chain carboxylate without metal co-ordination.

The 37 Hz coupling between the amide carbon and the tin nucleus and its remarkable downfield shift in the complex (Fig. 3A) show the co-ordination of dimethyltin(IV) to the deprotonated amide nitrogen. The neighbouring CH carbon exhibits also a scalar coupling with tin of 18–19 Hz.

On the basis of the potentiometric and NMR results, Gly-Asp is bound to dimethyltin(IV) by a $\{CO_2^-,N^-,NH_2\}$ donor set. The deprotonation of ML leading to $MH_{-1}L$ can be attributed to the co-operative proton loss of the amino and amide nitrogens followed by a water release from the co-ordination sphere of the cation. Eqn. (3) may give a better description for

Table 2 ^{1}H NMR chemical shifts in ppm and coupling constants in Hz (in parentheses) of complexes with dimethyltin(IV) in aqueous solution at pH 7.15 (Gly-Asp) and 6.84 (Asp-Gly). [Gly-Asp] = [Asp-Gly] = 2[$(CH_3)_2Sn^{IV}$] = 0.01 mol dm^{-3}



Species	δ_1	δ_3	δ_5	$\delta_{Sn(CH_3)_2}$ ($^2J_{Sn-H}$)
$(CH_3)_2Sn^{IV}$/Gly-Asp	3.542, 3.422 (17.0)[a]	4.190	2.867, 2.778 (15.9)[a]	0.839 (83.3)
$MH_{-1}L$ complex	$^3J_{CH_2-NH_2}$ (8.2, ≈1.5; 8.2)	$^3J_{CH-CH_2}$ (4.7, 3.5)	$^3J_{CH_2-CH}$ (4.7, 3.5)	0.663 (77.8)
	$^3J_{H-Sn}$ (12.0)[b]	$^3J_{H-Sn}$ (28.2)		
"free" ligand or "free" cation[c]	3.761	4.413	2.687, 2.445 (15.9)[a]	0.642 (82.5)
		$^3J_{CH-CH_2}$ (3.8, 10.3)	$^3J_{CH_2-CH}$ (3.8, 10.3)	
$(CH_3)_2Sn^{IV}$/Asp-Gly	3.786	3.825, 3.788 (18.9)[a]	2.648	0.813 (82.2)
$MH_{-1}L$ complex	$^3J_{CH-CH_2}$ (5.3)		$^3J_{CH_2-CH}$ (5.3)	0.771 (81.6)
	$^3J_{CH-NH_2}$ (≈5.3)			
"free" ligand or "free" cation[c]	4.180	3.840, 3.650 (17.5)[a]	2.763, 2.641 (17.0)[a]	0.643 (82.5)
	$^3J_{CH-CH_2}$ (4.7, 8.8)		$^3J_{CH_2-CH}$ (4.7, 8.8)	

[a] AB case. [b] Coupling between one of the AB type hydrogens and tin. [c] In the solution containing the dipeptide and the dimethyltin(IV) cation.

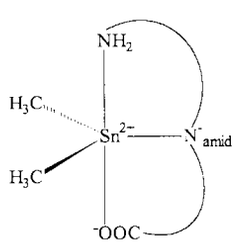
Table 3 ^{13}C NMR chemical shifts in ppm and ^{13}C–Sn coupling constants in Hz (in parentheses) with or without dimethyltin(IV) in aqueous solution at pH 7.34 (Gly-Asp) and 6.82 (Asp-Gly). [Gly-Asp] = [Asp-Gly] = 2[$(CH_3)_2Sn^{IV}$] = 0.15 mol dm^{-3} (without metal ion: [Gly-Asp] = [Asp-Gly] = 0.05 mol dm^{-3})[a]

Species	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	$\delta_{Sn(CH_3)_2}$
$(CH_3)_2Sn^{2+}$/Gly-Asp	43.93	174.60	54.34	180.74	40.17	179.18	1.44
$MH_{-1}L$ complex		(^2J = 37.0)	(^2J = 18.7)	(^2J ≈ 7)			(^1J = 647.8)
							−0.08
							(^1J = 627.2)
"free" ligand or "free" cation	41.86	168.09	54.18	179.16	40.41	179.62	3.34
$(CH_3)_2Sn^{2+}$/Asp-Gly	53.21	176.45	46.56	178.77	39.68	178.79	1.79
$MH_{-1}L$ complex		(^2J = 34.5)	(^2J = 21.3)	(^2J ≈ 8)		178.55[b]	(^1J = 657.7)
				178.74[b]			0.69
							(^1J = 651.1)
"free" ligand or "free" cation	52.05	170.65	44.34	177.16	38.16	177.18	3.59
				177.01[b]		176.91[b]	

[a] Owing to the fused chelate rings formed in the complexes, a contribution of other couplings (^3J(C2) or ^4J(C3, C6) in the case of Gly-Asp and ^3J(C2, C3, C4) or ^4J(C6) in case of Asp-Gly) to the reported J values cannot be fully discarded. [b] Chemical shifts from the spectrum measured at pH 5.1.

$$[(CH_3)_2Sn(HL)(OH)] \rightleftharpoons [(CH_3)_2SnH_{-1}L]^- + H^+ + H_2O \quad (3)$$

the whole process explaining the observed one equivalent base consumption. The angle between the two methyl groups of dimethyltin(IV), determined from the $^2J(^{119}Sn-^1H)$[30] and $^1J(^{119}Sn-^{13}C)$ couplings,[31] is about 128–135°, suggesting a trigonal bipyramidal structure for $MH_{-1}L$ where the methyl groups are in equatorial positions. To have more information about the structure and geometry of the complexes Mössbauer spectra were also recorded in quick-frozen solution. These results are discussed below.



NMR information for the co-ordination mode of Asp-Gly

The signals of the CH_2 hydrogens in $MH_{-1}L$ are considerably shifted as compared with those of the "free" ligand. The fine structure of the NMR patterns is also affected by the complexation (Table 2 and Fig. 2D). The strong upfield shift observed at pH 6.84 for the hydrogens belonging to the chiral carbon and the fact that the chemical shift is similar to that observed for the "free" ligand when the amino group is not protonated suggests that the amino group is bound to the dimethyltin(IV) cation. The CH hydrogen in question is coupled to the NH_2 hydrogens as revealed by a 2-D COSY experiment (see Fig. S1 in supporting information) and the presence of this coupling is an indirect proof of the co-ordination of the NH_2 group. The coupling pattern is simplified when the solvent is D_2O, *i.e.* the amino protons are substituted by deuterium nuclei (Fig. 2E). Beside the above facts, the pH-metric data also support indirectly the binding of the amino group to the $(CH_3)_2Sn^{IV}$ cation, since no deprotonation was detected around pH 8, where the amino group deprotonates in the "free" ligand.

Evidence for the co-ordination of other donor groups (carboxylate and amide) can be obtained from the ^{13}C NMR measurements. The chemical shifts of the two carboxylate groups of the "free" ligand are almost equivalent at pH ≈ 6.8 (the difference is 0.02 ppm). Above pH 7 one of these signals suffers a dramatic downfield shift in parallel with the deprotonation of the ammonium group. We may assume a stronger effect of the protonation state of the amino group on the chemical shift of the side chain carboxylate, which provides the possibility for assigning the carboxylate signals. In the presence of dimethyltin(IV) the pH dependent ^{13}C measurements showed a continuous chemical shift change as a function of pH for one of the carboxylate peaks in the complex while the chemical shift of the other is independent of the pH. ^{13}C NMR measurements with selective decoupling of the CH_2 hydrogens of the side chain (at 1060 Hz) resulted in a doublet structure for the carboxylate signal at higher field and a triplet structure for the one at

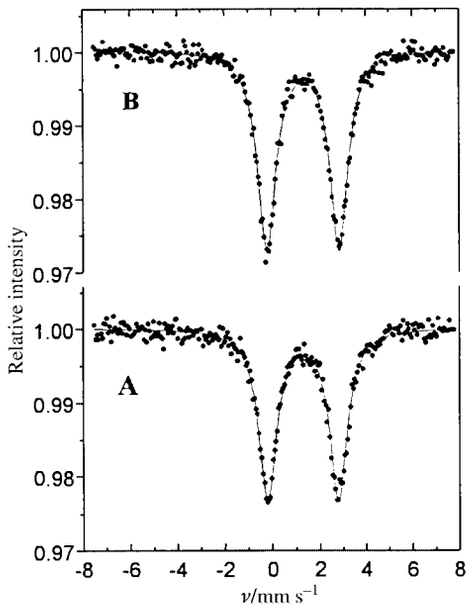


Fig. 4 "Quick-frozen" Mössbauer spectra of the $MH_{-1}L$ species formed in the dimethyltin(IV)–Gly-Asp (A) and –Asp-Gly (B) systems (pH 7.2, [L] = 0.2 mol dm^{-3}, [$(CH_3)_2Sn^{IV}$] = 0.1 mol dm^{-3}).

lower field. The signal having a triplet structure belongs to the C-terminal carboxylate group, for which the broad band proton decoupled ^{13}C spectrum shows clearly a three bond hetero-nuclear coupling with the tin nucleus (Fig. 3B). Consequently, the carboxylate signal at higher field belongs to the side chain group and it shows no coupling with tin.

The co-ordination of the amide group to dimethyltin(IV) is strongly supported by the observation of couplings with the tin nuclei: for the CON_{amide} carbon (shifted strongly downfield) with a coupling constant = 34.5 Hz (Fig. 3B) and for the $N_{amide}CH_2$ carbon ($^2J_{CH_2Sn}$ = 21.3 Hz). Finally, two signals appear for the methyl groups of the metal ion, although the inequivalency is not as characteristic as in the case of Gly-Asp. The $^2J(^{119}Sn–^1H)$ and $^1J(^{119}Sn–^{13}C)$ coupling constants can be converted into a ≈133° bond angle between these methyl groups. In this way the potentiometric and NMR results suggest the same structure and the participation of the same donor groups in the $MH_{-1}L$ species as those reported for Gly-Asp.

According to the distribution curves shown in Fig. 1, above pH 8, the hydrolytic species become dominant in the solution in the case of both peptides. This is consistent with the ^1H NMR spectra recorded at higher pH (see Fig. S2 in supporting information).

Mössbauer spectroscopic studies

To obtain more evidence for the co-ordination mode of the two dipeptides and for the structure of the species formed at neutral pH, Mössbauer spectroscopic measurements have been performed in quick-frozen aqueous solutions at pH 7.2. Owing to the relatively low sensitivity of this method, high metal and ligand concentrations have been used (see Fig. 4) similar to those of the ^{13}C NMR measurements. The spectra recorded can be well described by the presence of one species in solution in both systems which is in good agreement with the species distribution curves, calculated for the conditions used. The determined spectroscopic data and the calculated QS values for the $MH_{-1}L$ complexes are listed in Table 4. All parameters are very similar in the two systems studied, which strongly suggests identical co-ordination geometry for the central dimethyltin(IV) cations. The determined QS values are almost identical to the one found for the Gly-His complex of dimethyltin(IV) around neutral pH[15] and similar to those found for the $MH_{-1}L$ complex of Gly-Gly in different cases.[13,15] These facts provide further support for the trigonal bipyramidal {$2CH_3$, CO_2^-, N^-, NH_2} structure.

Table 4 Experimental Mössbauer parameters and calculated QS values of the $MH_{-1}L$ species formed in the two dimethyltin(IV)–dipeptide systems

Parameter	Gly-Asp	Asp-Gly
IS/mm s^{-1}	1.29	1.31
QS_{exp}/mm s^{-1}	3.00	3.06
Γ/mm s^{-1}	0.92	0.93
QS_{calc}/mm s^{-1}	2.78	2.78

Conclusion

Detailed studies concerning the co-ordination behaviour of two dipeptides, having a carboxylate group in their side chain, towards dimethyltin(IV) ions in aqueous solution provided new evidence for the deprotonation and co-ordination of the peptide nitrogen at a remarkably low pH. The carboxylate groups were able to act efficiently as anchoring donor groups for the amide deprotonation, although the side chain groups were released in parallel with the formation of the stable $MH_{-1}L$ species, having a similar {CO_2^-, N^-_{amide}, NH_2} donor set. The constitutional differences of Gly-Asp and Asp-Gly result in different stability and structure in the case of the complexes MHL and ML. Moreover, the extra stabilization in ML offered by Gly-Asp results in a 0.5 log unit higher pK for the ML $\rightleftharpoons$ $MH_{-1}L + H^+$ process as compared with the Asp-Gly complexes. The pK values of the copper(II) promoted amide deprotonation of Gly-Asp and Asp-Gly are 4.76 and 4.87, respectively.[32,33] This indicates a reverse order of stabilization in the ML complexes, *i.e.* provides further argument that the C-terminal carboxylate is the primary anchor in the case of dimethyltin(IV). The above mentioned co-ordination in $MH_{-1}L$ is so stable that side chain donor groups of Gly-Asp, Asp-Gly or even Gly-His[15] are not able to influence this structure.

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