Interaction of Dimethyltin(IV)$^{2+}$ Cation with Gly-Gly, Gly-His, and Some Related Ligands. A New Case of a Metal Ion Able To Promote Peptide Nitrogen Deprotonation in Aqueous Solution

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Equilibrium (pH-metric) and spectroscopic (^{1}H,^{13}C, and ^{119}Sn NMR and ^{119}Sn Mössbauer) studies were performed to characterize the interaction of the dimethyltin(IV) cation with glycine, glycyl-glycine (Gly-Gly), imidazole-4-acetic acid, histamine, histidine, glycyl-histamine, glycyl-histidine (Gly-His), and β-alanyl-histidine (carnosine). For histamine and glycyl-histamine (having only nitrogen donor atoms) no complex formation was detected. The hydrolyzed species of the dimethyltin(IV) cation are always dominant over the complexes formed with the other ligands, except with Gly-Gly and Gly-His. For these two ligands, {COO^-,N^-,NH_2} coordinated complexes are dominant in the neutral pH range with a trigonal bipyramidal structure, providing the first example that alkyltin(IV) cations are able to promote the deprotonation of the peptide-nitrogen in aqueous solutions, at unexpectedly low pH. In this process the carboxylate is the anchoring group (assisting by chelate formation), in contrast with any other metal ions which are known to coordinate to amide nitrogen. The metal coordination of the imidazole ring, which is suggested as binding site toward alkyltin(IV) cation in several proteins, was not observed for Gly-His under the conditions used; it is probably the case for the other ligands, too.

Introduction

The exponential increase of the industrial, agricultural and biological applications of the organo-tin(IV) compounds during the last 50 years has led to their accumulation in the environment and finally in biological systems. However, these compounds are generally very toxic even at low concentration. To obtain better insight into the biological action of alkyltin chemicals, first their speciation in biological systems must be known. The interaction of the organotin cations with different compounds present in biological fluids has received considerable importance only in the last years.[1−15] For example, $(Me)_2Sn^{IV}$ or $(Et)_3Sn^{IV}$ compounds have been shown to bind to rat hemoglobin through a deprotonated thiol group and an imidazole nitrogen.[1−3] Also, imidazole nitrogen(s) was suggested as binding site(s) of the trialkyltin(IV) cation to mitochondrial ATPase.[4] Such studies, however, concerning organotin binding to biological macromolecules are still very rare in the literature and they are far from the quantitative description. Low molecular weight compounds present in biological fluids may also bind to organotin cations, significantly altering their speciation. The characterization of these interactions can be also used for modeling the organotin binding to macromolecular compounds. Another demand for such solution equilibrium studies raised from the potential pharmaceutical application of organotin compounds. Today, a number of dialkyltin derivatives are known to have an efficient anticancer activity and their structure are well characterized in the solid state.[13−15] However, many questions may rise about their active forms in the living organisms (they may decompose, form mixed ligand complexes, etc.).

Recently, several sets of equilibrium data were published concerning dialkyltin(IV) complexes.[5−12] These studies revealed the "chameleon" nature of the dialkyltin(IV) cation, since strong

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affinity was reported toward ligands containing {O},[5−8] {S,O,N},[9] or {O,N}[9−12] donor sets.

Peptides, particularly those containing imidazole residue, are well-known as metal binding ligands in biological systems.[16−19] Several alkyltin(IV) complexes of peptides have been prepared and studied in the solid state (or dissolved in different solvents),[20−23] e.g. methyl *N*-benzoylleucyl-histidine was found to be a useful model to mimic alkyltin(IV) binding of proteins through imidazole nitrogen. The metal binding of amide nitrogen(s) is particularly important in peptide complexes. Although the X-ray diffraction study of some crystalline dialkyltin(IV)−peptide complexes provided definite evidence of the formation of Sn−N$^-$ bond,[1,21] its formation in aqueous solution is still questionable. Recently, we reported evidence for such a binding mode in case of the pseudo-peptide *N*-D-gluconyl-glycine,[11] but the polyhydroxy chain present in this molecule may act as an anchoring group in respect to the metal-promoted deprotonation of amide nitrogen, which prevents the direct comparison with peptide ligands. However, to our knowledge, no equilibrium data have been published concerning alkyltin(IV)−peptide systems.

The present paper describes the equilibrium (pH-metric) and spectroscopic (^{1}H, ^{13}C, and ^{119}Sn NMR and ^{119}Sn Mössbauer) properties of dimethyltin(IV) complexes of glycine, glycyl-glycine (Gly-Gly), imidazole-4-acetic acid, histamine, histidine, glycyl-histamine, glycyl-histidine (Gly-His), and β-alanyl-histidine (carnosine). The binding ability of different donor groups present in the above ligands—with special attention to amide and imidazole nitrogens—and the possible structure of the formed complexes are also discussed.

Experimental Section

Materials. Glycyl-histamine was prepared as described earlier.[18] All other chemicals, glycine and glycyl-glycine (Reanal), dimethyltin(IV) dichloride (Fluka), imidazole-4-acetic acid (Fluka), histamine (Aldrich), histidine, glycyl-histidine (Fluka), and β-alanyl-histidine (Fluka), were used without further purification. Every 2 days a new dimethyltin(IV) dichloride solution was prepared and standardized by acid−base titration.

pH-metric Measurements. The protonation and coordination equilibria were investigated by potentiometric titrations in aqueous solution (0.1 mol dm^{-3}, $NaClO_4$, and $T = 298 \pm 0.1$ K) using an automatic titration set including a Dosimat 665 (Metrohm) autoburet, an Orion-710A precision digital pH-meter and an Orion Ross model 8103BN combined glass electrode. The species formed in the systems were characterized by the following general equilibrium process

$$pM + qL + rH \leftrightarrow M_pL_qH_r$$

(where M denotes the dimethyltin(IV) cation and L the nonprotonated ligand molecule). Charges are omitted for simplicity but can be easily calculated by taking into account the following notation for the fully protonated ligands: glycine (LH_2), glycyl-glycine (LH_2), imidazole-4-acetic acid (LH_2), histamine (LH_2), glycyl-histamine (LH_2), histidine (LH_3), glycyl-histidine (LH_3), and β-alanyl-histidine (LH_3). The detailed description of the experimental procedure and data evaluation were described earlier.[8−11,18−19]

The protonation and the complex formation constants were determined from 4 and 8 independent titrations (80−110 data points per titration), respectively. The metal-to-ligand ratios varied between 1:1 and 1:6, using a dimethyltin(IV) concentration ranging from 2×10^{-3} to 1×10^{-2} mol dm^{-3}. The pH-metric data between pH = 2 and 10.5 were used for the evaluation.

NMR Measurements. ^{1}H, ^{13}C, and ^{119}Sn NMR measurements were performed at 400, 100.6, and 149.1 MHz, respectively, on a spectrometer Bruker DRX400.

For ^{1}H and ^{13}C spectra, the chemical shifts δ were measured with respect to dioxane as an internal reference and converted relative to the TMS chemical shift scale using the following values: $\delta_{dioxane} = 3.70$ ppm for ^{1}H and 67.4 ppm for ^{13}C NMR. ^{13}C peak assignments for bound molecules (particularly the CO signals) were made by an Attached Proton Test experiment (equivalent to a DEPT experiment) and by 2D ^{13}C−^{1}H correlation experiments. For the ^{119}Sn NMR experiments ($I = {}^1/_2$, 8.69%), $Sn(CH_3)_4$ in $CHCl_3$ was used as an external reference ($\delta = 0$ ppm). ^{119}Sn{^{1}H} spectra were recorded using the gate decoupling technique in order to prevent negative NOE effects.

For the calculation of the individual chemical shifts and ^{1}H−Sn coupling constants of the different hydrolyzed species of the dimethyltin cation, the ^{1}H NMR specta of the dimethyltin(IV) solution were recorded at 16 different pH values between 2 and 12. The corresponding concentrations of the hydrolyzed species at any pH were calculated by using the known hydrolysis constants. The individual NMR parameters (δ, J) belonging to each hydrolyzed species (assuming fast mutual exchange) were calculated using a nonlinear parameter fitting program. The individual $^2J(^{119}Sn-^1H)$ values, determined in this way, could be converted to C−Sn−C angles using the published equation.[24]

For ^{1}H NMR measurements, the ligand concentration was 0.045 (Gly-Gly) or 0.020 (Gly-His) and the metal concentration 0.015 mol dm^{-3}. In the case of ^{13}C NMR, the concentrations used were 0.18 and 0.06 mol dm^{-3} for the ligand and for the metal, respectively. For ^{119}Sn NMR the concentrations of the ligand and the cation were 0.030 and 0.025 mol dm^{-3}, respectively.

Generally, measurements were 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 obtained with $Ca^{119}SnO_3$ (10 mCi, Radiochemical Centre, Amersham, U.K.) source moving at room temperature. The absorber samples of the Me_2Sn^{2+} derivatives were measured in cylindrical polyethene sample holders ($\cong$1 mL, 1 cm^2 section, corresponding to 0.025−0.050 mg ^{119}Sn/cm^2) and maintained at liquid nitrogen temperature in a model NRD-1258-MB (Cryo, USA) cryostat. The 77.3 ± 0.1 K temperature was controlled through a model ITC-2 temperature controller from Oxford Instruments (Oxford, England).

The source motion was effected by the following Wissenschaftliche Elektronik GmbH apparatus (Germany): a velocity transducer (range 0 to ±10 mm s^{-1}), an FG-2 function generator, and an MR 250 driving unit. Velocity calibration was carried out with an enriched iron foil spectrum (^{57}Fe = 99.99%, thickness 0.06 mm, Dupont, MA) at room temperature, using a ^{57}Co source (10 mCi, Dupont, MA) in a Palladium matrix. The zero point of the Döppler velocity was determined at room temperature, through absorption spectra of natural $CaSnO_3$ containing 0.5 mg/cm^2 of ^{119}Sn. Finally, a model 269 multichannel analyzer (Takes, Ponteranica, Bergamo) was used and 5×10^5 counts were collected for each velocity point. The obtained data were refined with the appropriate software to obtain the Mössbauer parameters isomer shift, IS (mm s^{-1}), nuclear quadrupole splitting, QS (mm s^{-1}), and the width at half-height of the resonant peaks, Γ (mm s^{-1}), reported in Table 3. The comparison of the experimental QS values with those calculated on the basis of the point-charge model formalism[25] enabled us to determine the steric arrangements of the complex species formed. The partial quadrupole splitting (PQS) values of the different functional groups used in calculations were taken from the literature.[2,26] The PQS

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Table 1. Formation Constants and Derived Data for Metal Complexes of Studied Ligands (as their logarithms), $I = 0.1$ mol dm^{-3} ($NaClO_4$), $T = 298$ K, $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$, with Estimated Errors in Parentheses (Last Digit)[a]

log β_{pqr}	glycine	imidazole-4-acetic acid	histidine	Gly-Gly	Gly-His	carnosine
011	9.50(1)	7.336(2)	9.082(2)	8.089(2)	8.146(2)	9.397(2)
012	11.88(1)	10.524(2)	15.102(2)	11.209(3)	14.879(2)	16.193(2)
013	–	–	16.809(4)	–	17.406(2)	17.493(2)
112	–	–	16.25(5)	–	17.16(7)	17.54(3)
111	11.03(3)	9.08(5)	13.23(3)	10.07(1)	13.73(5)	14.37(2)
110	7.99(2)	5.51(3)	7.96(4)	6.61(1)	9.05(2)	8.32(2)
11−1	2.40(3)	−0.08(4)	1.56(5)	1.80(1)	2.56(1)	1.73(2)
log K^b	1.53	1.74	1.15	1.98	2.28	1.35
pK_1	3.04	3.57	3.02	3.46	3.43	3.17
pK_2	5.59	5.59	5.27	4.81	4.68	5.45
pK_3	–	–	–	–	6.49	7.19
experimental points	420	276	587	586	440	564
fitting parameter	0.006	0.004	0.005	0.006	0.005	0.006

[a] The hydrolysis constants of the dimethyltin(IV) ion are the following: log $\beta_{10-1} = -3.175(5)$, log $\beta_{10-2} = -8.415(4)$, log $\beta_{10-3} = -19.459(4)$, log $\beta_{20-2} = -4.95(4)$, and log $\beta_{20-3} = -9.96(3)$. [b] log K refers to the stability constant of the carboxylate coordinated complexes (log K = log β_{11n} − log β_{01n}, where $n = 1$ and 2).

value for the water-oxygen atom in octahedral structure $[H_2O]^{oct}$ has been calculated as 0.09 mm s^{-1} from Mössbauer measurements performed for dimethyltin(IV) aquocation, the presence of which has been evidenced in all our systems in glassy state (the corresponding literature value reported as 0.20 mm s^{-1} by Barbieri et al.,[26] which has been calculated[27] from $[H_2O]^{tba} = 0.18$ mm s^{-1}).

Results and Discussion

Hydroxo Complexes. Dimethyltin(IV) cation is known[5,6,8] to form stable and water soluble mono- and polynuclear hydroxo species (see Table 1) in the whole pH-range studied. Since the hydroxide ion and the studied ligands are in strong competition for the metal ion, these species were always taken into consideration in the equilibrium systems. The ^{1}H NMR spectrum of dimethyltin(IV) chloride solutions presents a sharp signal with satellite peaks, as a result of heteronuclear couplings (^2J(^{117}Sn−^{1}H) and ^2J(^{119}Sn−^{1}H)) with the two NMR active isotopes of tin (7.6% of natural abundance for ^{117}Sn and 8.69 for ^{119}Sn). Both the chemical shift and the coupling constants decrease with increasing pH. The ^2J(^{119}Sn−^{1}H) values can be used to determine the C−Sn−C angle, providing information on the structure of the species formed.[24] On the basis of the ^{1}H NMR spectra of dimethyltin(IV) chloride solutions recorded at different pH—taking into account the known species distribution of hydroxo complexes being in fast mutual exchange—it is possible to calculate the individual NMR parameters (δ, ^2J) belonging to the different species (see Experimental Section). The calculated values for the different hydrolytic species are the following: M ($\delta = 0.89$ ppm, ^2J(^{119}Sn−^{1}H) = 106 Hz), MOH (0.87, 95), $M(OH)_2$ (0.64, 81), $M(OH)_3$ (0.41, 81), $M_2(OH)_2$ (0.74, 80), and $M_2(OH)_3$ (0.79, 82). These data suggest an octahedral structure for the aqua ion ($\angle$C−Sn−C ∼ 175°), a trigonal bipyramidal (in case of $M(OH)_2$, more likely a tetrahedral[2]) structure for the complexes $M(OH)_2$, $M(OH)_3$, $M_2(OH)_2$ and $M_2(OH)_3$ ($\angle$C−Sn−C ∼ 132°), while in the case of MOH an intermediate value can be determined ($\angle$C−Sn−C ∼ 154°), probably as the result of (i) the coexistence of both octahedral and trigonal bipyramidal structures in fast mutual exchange or (ii) a very distorded stucture.

Complexes with the Studied Ligands. The determined protonation and formation constants together with some derived data are collected in Table 1, while some representative species distribution diagrams are depicted in Figure 1. In the case of the two imidazole-containing N-donor ligands which do not contain carboxylate group (histamine and glycyl-histamine), no interaction could be detected with the dimethyltin(IV) cation, showing the low coordination affinity in aqueous solution of this metal ion toward imidazole or amino groups, in the absence of other donor groups.

In the case of the other ligands, the complex formation processes start already at pH = 2 (Figure 1). The first complexes formed are MLH and MLH_2 for the ligands having two and three labile protons, respectively. The similar stability constants of these species (log $K \sim 1.7$ for the reactions M + LH = MLH or M + LH_2 = MLH_2, see Table 1) in all the studied systems are in favor of the monodentate carboxylate coordination in these complexes. It can be noted that the log K values do not correlate with the pK_a of the carboxylic groups indicating that the stability is affected by several effects (hydrogen bonds, possible interaction with the amide oxygen of peptides, hydrophobic interactions, etc.).

All these complexes suffer a deprotonation near pH 3−4, leading to the ML or MLH species. The pK values corresponding to these processes (p$K_1 \sim 3.2$, see Table 1), are again similar to each other in all systems, and may be attributed (i) to the deprotonation of a metal bound water molecule, forming hydroxo mixed-ligand complexes or (ii) to the metal promoted deprotonation of amino group. The analogous processes in the case of dimethyltin(IV) complexes of amino acids with non-coordinating side chains,[12] were assigned to the latter process. Our data are, however, inconsistent with this assumption: (i) despite the different basicity of the nitrogens in glycine and imidazole-4-acetic acid, the MLH = ML + H processes are characterized with similar pK values; (ii) in the case of the assumed $\{COO^-,NH_2\}$ coordination, a five-membered chelate may form with glycine, however, in the case of Gly-Gly, an eight membered one may form, which would result also in significantly different pK values; (iii) if the amino nitrogen deprotonates in this process, e.g., in case of glycine, the more than 6 logarithmic unit decrease of pK_{NH_3}, compared to the free ligand, would correspond to a very high affinity of dimethyltin(IV) cation toward amino donors, which was not detected in the case of histamine and glycyl-histamine. The above contradictions could be only explained by the formation of hydroxo mixed ligand complexes during the deprotonation in question. It is worth mentioning that the above complexes are always

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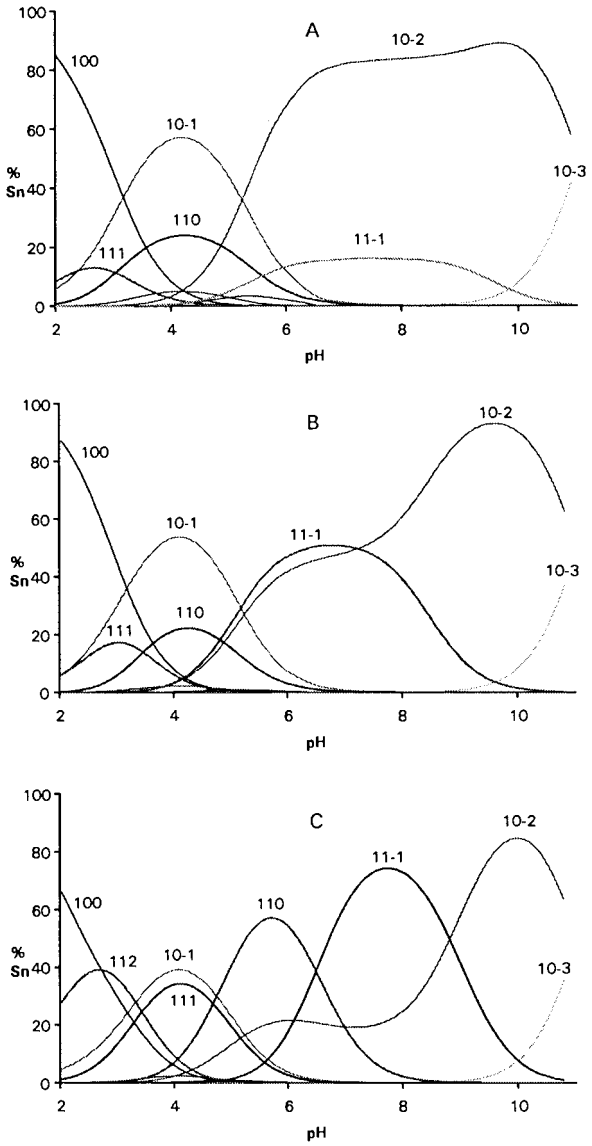


Figure 1. Species distribution curves in the dimethyltin(IV)−glycine (A), −Gly-Gly (B), and −Gly-His (C) systems. Hydrolytic species are shown by thin lines. For clarity, curves belonging to the $M_2(OH)_2$ and $M_2(OH)_3$ complexes (minor species between pH 3−5) are not labeled ([M] = 0.003 mol dm^{-3}, [L] = 0.01 mol dm^{-3}). The notation of the different species corresponds to the *pqr* values of the corresponding complex $M_qL_pH_r$.

minor species and that the hydrolysis of the dimethyltin(IV) cation is the dominant process (Figure 1).

It can be noted that the pK_1 values are rather similar to −log β_{10-1} values indicating that the hydrolyzed $Sn(CH_3)_2(OH)$ species has a comparable affinity for a carboxylate group as the aqua ion. That can be explained by the different structures of the aqua ion (octahedral) and the carboxylate coordinated species (trigonal bipyramidal, see later) undergoing water deprotonation.

A further deprotonation is observed between pH 4−6. In this case, however, the corresponding p*K* values of Gly-Gly and Gly-His (pK_2 = 4.81 and 4.68, respectively) are 0.5−0.9 log units smaller than those of the other ligands (pK_2 = 5.45 ± 0.15), resulting in a considerable difference for the speciation, the complex formation processes becoming dominant over the hydrolysis. Above pH = 10, in all the systems, only the hydrolytic species of dimethyltin(IV) cation are present.

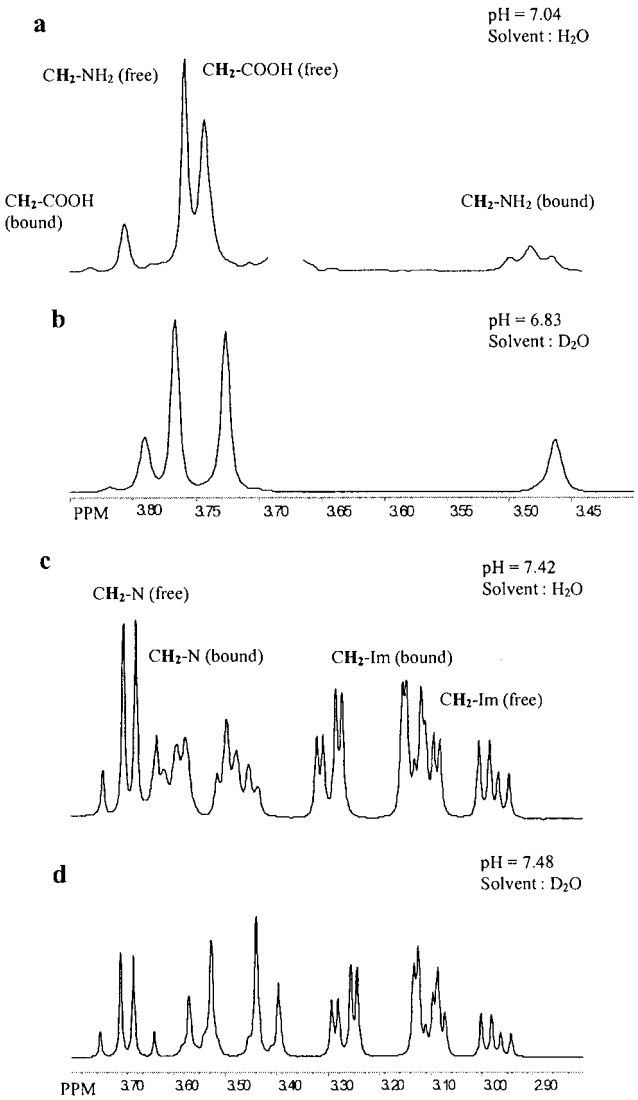
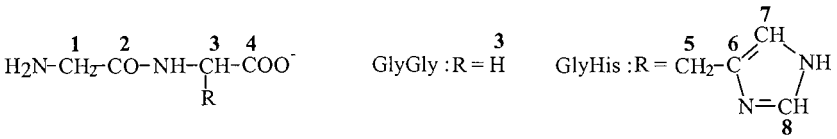


Figure 2. Part of the ^{1}H NMR spectra of the dimethyltin(IV)−Gly-Gly (a, b) and −Gly-His (c, d) systems in H_2O (a, c) or D_2O (b, d). For the concentration of the species, see the Experimental Section.

To have a better insight into the structures of the species formed, a multinuclear NMR study was performed. The NMR spectra of the investigated systems do not show any significant differences compared to the metal-free spectra of ligands between pH 2−4.5. In the dimethyltin(IV)−Gly-Gly and −Gly-His systems, however, new NMR peaks appear in the spectra (Figure 2), parallel to the formation of MLH_{-1} (in the case of Gly-Gly) or ML and MLH_{-1} (in the case of Gly-His, see later), indicating that the exchange between these complexes and the free ligand is slow relative to the NMR time scale. This behavior is not observed for the dimethyltin(IV)-containing systems with the other ligands; consequently, the species formed in the later systems are in fast mutual exchange. The above facts strongly support different deprotonation processes according to the case of the above-mentioned two groups of ligands which will be separately discussed in the following.

The deprotonation process in question ($ML = MLH_{-1} + H$ or $MLH = ML + H$), as for the precedent deprotonation, can be assigned again to two pathways: (i) the proton loss of a metal-bound water molecule or (ii) metal promoted deprotonation of the amino group. The similar p*K* values, despite the different amino nitrogen basicity, are again in favor of the metal-bound water deprotonation; however, these values (p$K_2 \sim 5.4$) are

Table 2. ^{1}H NMR Chemical Shifts in ppm and Coupling Constants in Hz (in Parentheses) of Dipeptides (Gly-Gly and Gly-His) Complexes with Dimethyltin Cation in Aqueous Solutions at pH = 7.04 (Gly-Gly), pH = 5.53 (Gly-His, Mainly ML), and pH = 7.40 (Gly-His, Mainly MLH_{-1})



	δ_1	δ_3	δ_5	δ_7	δ_8	$\delta_{Sn(CH_3)_2}$ ($^2J_{H-Sn}$)
$Sn(Me)_2^{2+}$/GlyGly						
complex MLH_{-1}	3.510 $^3J_{CH_2-NH_2} = 6.6$	3.828 $^3J_{H-Sn} = 22.5$	–	–	–	0.789 (82.2)
free ligand or free cation[a]	3.764	3.780	–	–	–	0.649 (82.2)
$Sn(Me)_2(OH)_x$[b]	–	–	–	–	–	0.647 (81.2)
$Sn(Me)_2^{2+}$/GlyHis						
pH = 5.53						
complex ML	3.585, 3.457[c]	(*d*)	3.353, 3.225[e (g)]	7.125	8.495	0.697[f] (82.2) 0.301[f] (79.7)
free ligand or free cation	3.788, 3.725[c]	(*d*)	3.187, 3.075[e (h)]	7.191	8.481	0.724 (85.6)
$Sn(Me)_2(OH)_x$[b]	–	–	–	–	–	0.730 (84.6)
pH = 7.40						
complex MLH_{-1}	3.583, 3447[c]	(*d*)	3.268, 3.118[e (g*)]	6.867	7.862	0.647[f] (80.2) 0.148[f] (80.7)
free ligand or free cation	3.725, 3.668[c]	(*d*)	3.092, 2.971[e (h*)]	7.019	8.018	0.643 (81.4)
$Sn(Me)_2(OH)_x$[b]	–	–	–	–	–	0.625 (80.4)

[a] Free ligand or free cation in the solution containing the dimethyltin cation and the dipeptide. [b] Dimethyltin cation aqueous solution without ligand at the same pH value. [c] Two inequivalent hydrogens (AB case), $^2J_{H-H} = 16.1$ Hz. [d] Nondetectable because the presence of the water signal. [e] Two inequivalent hydrogens (AB part of a ABX case). [f] Inequivalent methyl signals. [g] $^2J_{H-H} = 15.2$ Hz, $^3J_{H-H} = 7.8$ and 5.4 Hz; and [g*] $^2J_{H-H} = 15.2$ Hz, $^3J_{H-H} = 8.3$ and 5.4 Hz. [h] $^2J_{H-H} = 15.6$ Hz, $^3J_{H-H} = 7.8$ and 5.4 Hz; and [h*] $^2J_{H-H} = 15.2$ Hz, $^3J_{H-H} = 8.3$ and 4.9 Hz.

Table 3. ^{13}C NMR Chemical Shifts in ppm and ^{13}C−Sn Coupling Constants in Hz (in Parentheses) of Dipeptides (Gly-Gly and Gly-His) Aqueous Solutions with or without Dimethyltin Cation at pH = 7.10 (Gly-Gly) and pH = 8.10 (Gly-His)[a]

	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8	$\delta_{Sn(CH_3)_2}$
$Sn(CH_3)_2$Gly-Gly									
complex	43.89	174.74 ($^2J = 34.9$)	46.31 ($^2J = 20.7$)	178.50 ($^2J \sim 11$)	–	–			1.30 ($^1J = 656.9$)
free ligand or free cation	44.17	168.39	41.73	177.31	–	–	–	–	3.25
$Sn(CH_3)_2$Gly-His									
complex	44.03	174.67 ($^2J = 36.2$)	56.02 ($^2J \sim 14$)	179.83 ($^2J = 11$)	28.80	132.53	119.26	136.52	0.59[b] ($^1J = 646.6$)
	–	–	–	–	–	–	–	–	−0.22[b] ($^1J = 663.4$)
free ligand or free cation	42.54	169.97	57.32	178.44	29.73	133.65	118.31	136.52	2.84

[a] Due to the fused chelate rings present in the complexes, a contribution of ^3J (carbons 2 and 3) or ^4J (carbon 4) to the reported *J* values cannot be fully discarded. [b] Inequivalent methyl signals.

much higher than in former case ($pK_1 \sim 3.2$), thus in the absence of the unambiguous additional information none of these possibilities can be definitely rejected.

The above coordination possibilities do not hold, however, for the corresponding complexes of Gly-Gly and Gly-His, as our NMR study showed. First of all, generally none of the above-mentioned coordination modes would result in species being in slow exchange. Two sets of peaks are, however, observed in the NMR spectra of ligands (^{1}H, ^{13}C) between pH = 4−10 and those of the dimethyltin(IV) cation (^{13}C, ^{119}Sn). The full assignment of the observed ^{1}H and ^{13}C NMR peaks (together with some coupling constants) are collected in Table 2 and Table 3, respectively. Two sets of NMR peaks were also reported, when crystalline $(CH_3)_2Sn^{IV}$−Gly-Gly[22] or −Gly-His[23] complexes were dissolved in aqueous solution. One of them was assigned to the crystallographically characterized {COO^-, N^-,NH_2} coordinated species, while the other to a partially hydrolyzed complex, in which the metal ion is coordinated monodentately through the amide nitrogen. Combining our data provided by the pH-metric results and by the pH dependence of the NMR spectra of the free ligand, the metal and the metal−ligand systems, one set of peaks belonging to the (i) nuclei of the ligands can be unambiguously assigned to the noncomplexed ligand and (ii) in the case of nuclei of the metal ions to the hydrolyzed species (mostly to the $(CH_3)_2Sn(OH)_2$ species). The other sets belong to the pH-metrically observed MLH_{-1} (Gly-Gly) or ML and MLH_{-1} (Gly-His) complexes. No experimental data supported the formation of the above-mentioned[22] partially hydrolyzed species, which in fact, would be extremely unstable in aqueous solution.

Our NMR data provided the following additional information.

(a) The observed chemical shifts for the MLH_{-1} complexes, are nearly coincident with those reported for the corresponding $(CH_3)_2Sn^{IV}$−Gly-Gly[22] (^{1}H, ^{13}C) or −Gly-His[23] (^{1}H) crystalline complexes dissolved in aqueous solution (although the authors did not provide full assignment of the peaks). These facts, as well as the observed $^2J(^{119}Sn-^1H)$ and $^1J(^{119}Sn-^{13}C)$ coupling constants (see Tables 2 and 3), strongly support the formation of the same structure in the presently formed MLH_{-1} species, as already reported for the solid-state complexes based on X-ray diffraction studies,[1,14,21] namely, {COO^-,N^-,NH_2} coordination in a trigonal bipyramidal structure. A pair of signals were detected for the Sn−CH_3 groups in the kinetically stable

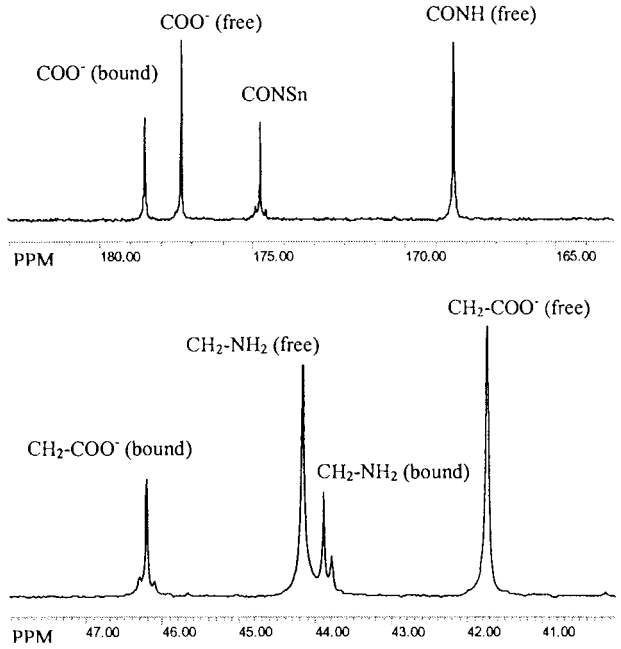


Figure 3. Part of the ^{13}C NMR spectrum of the dimethyltin(IV)−Gly-Gly system at pH = 7.1 ([Sn]/[ligand] ratio equal to 3). The upper spectrum is relative to the CO region, and the lower one, to the CH_2 region. Couplings between the tin and the ^{13}C nuclei are observed for COO^- (bound), CONSn, and CH_2-COO^- (bound).

complexes of Gly-His (Tables 2 and 3), similarly as it was already reported,[23] due to the presence of a chiral center in the molecule.

(b) In the $(CH_3)_2Sn^{IV}$−Gly-His system, both pH-metric and NMR results show also the formation of the ML species between pH 4−8. Despite the kinetically stable nature of the MLH_{-1} (and ML) complex of Gly-His, the $ML = MLH_{-1} + H^+$ deprotonation is characterized by fast NMR site exchange (e.g. the imidazole protons continuously shifted to the upper field during this process and, also, only one set of peaks is detected for ML and MLH_{-1}). This indicates a deprotonation on a distant group from the metal ion, which is not involved in the coordination. Indeed, the pH dependence of NMR spectra suggests the proton loss of the nonbound imidazole ring; furthermore, the pK value of the above process (pK_3 = 6.49) is close to that of the imidazole nitrogen in the free ligand (pK = 6.73). As a conclusion, the coordination mode and geometry are the same in ML and MLH_{-1} complexes of Gly-His.

(c) Several (still not reported) coupling constants were observed, which may furnish further evidences for the above proposed $\{COO^-,N^-,NH_2\}$ coordination: (i) the slow NMR site exchange of the ML and MLH_{-1} complexes with the free ligands, prevents the fast proton-exchange between water molecules and the coordinated terminal amino group in the complex. This results in a detectable coupling between these amino protons and those of the next methylene group in the complex, leading to the observations of a coupling pattern in the case of Gly-Gly and Gly-His, respectively (Figure 2a and c). This is proof for the coordination of the amino group. In D_2O solution, this coupling obviously disappears (Figure 2b and d); (ii) on the ^{13}C NMR spectra of both systems, couplings of the ^{13}C nuclei with ^{117}Sn and ^{119}Sn nuclei were observed for the amide ($J \sim 35-36$ Hz) and the carboxylate carbon ($J \sim 10-11$ Hz), and also for the methylene carbon in the case of Gly-Gly (Table 3, Figure 3) and for the CH carbon in Gly-His system. (iii) on ^{1}H NMR spectra, couplings are observed between the hydrogens of the CH_2-COO^- group and tin for the Gly-Gly system ($J \sim 22$ Hz) and between the CH hydrogen and tin for the Gly-His system ($J \sim 28$ Hz). For this latter system a weak coupling is also observed for one of the two H of the CH_2-amino group with tin ($J \sim 9-10$ Hz); the corresponding coupling for Gly-Gly is not observed probably as the consequence of broader peaks for this system than for the other.

To sum up our NMR study, the results prove the formation of a $\{COO^-,N^-,NH_2\}$ coordinated species for both Gly-Gly and Gly-His, having trigonal bipyramidal structures, which are identical with those reported earlier by X-ray diffraction in the solid state. The above results provided the first example for the formation of the alkyltin(IV)−peptide amide bond in aqueous solution.

Despite the results obtained for the Gly-His complexes, in case of the carnosine (having identical donor groups) the $\{COO^-,N^-,NH_2\}$ coordination was not observed. This fact shows that the fused (6,5) membered chelate, which would form in case of carnosine (fused (5,5) membered chelate forms with Gly-His), cannot stabilize the amide coordinated complex.

It is worth mentioning that, on the basis of our results, the formation of e.g. the MLH_{-1} species of Gly-Gly can be formulated as $M(LH)(OH) = MLH_{-1} + H^+ + H_2O$. Thus the deprotonation of the amide and amine nitrogens takes place in a cooperative manner with the removing of the coordinated hydroxy group. Similar transformation was reported in the case of diethyltin(IV) complexes of *N*-D-gluconyl-glycine,[11] concerning the cooperative deprotonation of amide and alcoholic OH groups. The overall pK value of the above process in the latter case (pK = 4.46) is close to the presently determined pK values (pK = 4.81 and 4.68 for Gly-Gly and Gly-His). Taking into account, however, the considerably higher pK values of OH group[28] compared to that of the RNH_3^+ group, the above values show higher affinity of dialkyltin(IV) cation to form $\{COO^-, N^-,O^-\}$-than $\{COO^-,N^-,NH_2\}$-coordinated species.

Two important features of dimethyltin(IV) cation should be mentioned comparing the above finding with the ability of other metal ions to promote ionization of amide nitrogen: (i) the metal-promoted deprotonation of amide nitrogen in the presently investigated systems occurs at surprisingly low pH (= 4−5). Very few metal ions are able to bind amide nitrogen at such a low pH, and all of them have "soft" character, e.g. Pd(II) and Cu(II).[16] In this context, the dimethyltin(IV) cation, generally considered as a "hard" acceptor, is a unique example; (ii) the metal ion-promoted deprotonation of amide nitrogen in peptides is always preceded by a coordination of the metal ion to another donor group(s) of the ligand (anchoring group), which assists through the formation of a chelate ring. In case of the metal ions featuring a strong ability to bind amide nitrogen, the anchoring group is always the N-terminal amino group (completed with the coordination of the carbonyl oxygen), as the consequence of their "soft" character. This picture does not hold, however, for the dimethyltin(IV) cation, as it was proved by the NMR results and by the characteristic differences between the dimethyltin(IV) binding of glycyl-histamine and glycyl-histidine. In this case, the carboxylate oxygen plays the role of anchoring group, which is again an unique behavior of this cation.

Mössbauer Spectroscopic Studies. To determine the geometry of the species discussed above, ^{119}Sn Mössbauer spectroscopic measurements were performed at selected pH values for the quick-frozen solution of the dimethyltin(IV)−glycine, −Gly-

(28) Burger, K.; Nagy, L. Metal complexes of carbohydrates and sugar-type ligands. In *Biocoordination Chemistry*; Burger, K., Ed.; Ellis Horwood: New York, 1990.

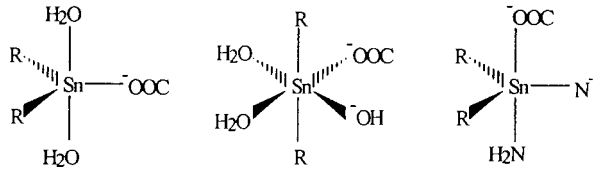


Figure 4. Schematic structure of the complex species.

Table 4. Experimental and Calculated Mössbauer Parameters of the Complex Species in Different Systems

ligand	species	IS (mm s^{-1})	$QS_{exp.}$ (mm s^{-1})	Γ (mm s^{-1})	$QS_{calc.}$ (mm s^{-1})	structure
glycine	MLH	1.30	3.58	0.76	3.57	a
	ML	1.42	4.38	0.84	4.27	b
Gly-Gly	MLH	1.26	3.54	0.83	3.57	a
	ML	1.36	4.37	0.89	4.27	b
	MLH_{-1}	1.18	3.35	0.80	2.78	c
Gly-His	MLH_2	1.49	3.73	0.76	3.57	a
	MLH	1.36	4.09	0.89	4.27	b
	ML	1.19	3.07	0.91	2.78	c
	MLH_{-1}	1.18	3.02	0.88	2.78	c

Gly, and −Gly-His systems. Due to the sensibility of this method, considerably higher concentrations ([M] = 0.05, [L] = 0.1 mol dm^{-3}) than for pH-metry were used. The limited solubility of the $M(OH)_2$ species, however, prevented the measurements above pH = 6.5 in the case of glycine and pH = 9.5 in the case of Gly-Gly. The experimental and calculated Mössbauer parameters are listed in Table 4.

The Mössbauer spectra for the three systems, measured in the strong acidic region, contain two overlapping doublets which can be assigned, on the basis of the concentration distribution curves to the hydrated metal ion (100) and to the MLH (111) complex for glycine and Gly-Gly or to MLH_2 (112) for Gly-His. The QS value found for the (100) species is very similar to that published by Barbieri and Silvestri[29] as a result of Mössbauer-monitored hydrolysis of dimethyltin(IV)$^{2+}$. The comparison of the experimental and calculated QS values indicates that the other species formed contains the coordinating carboxylate group as well as alkyl groups in the equatorial and slightly bounded water molecules in axial positions (Figure 4a).

On increasing pH the MOH, ML (Gly-Gly), and MLH (Gly-His) complexes are the major species in the studied systems, as also concluded from potentiometric measurements. The structure of the MOH complex is similar to the carboxylato-monocoordinated one, but the third equatorial position is occupied by a hydroxide ion instead of a carboxylate group. The experimental QS values for the ML (MLH in case of Gly-His) complex species are in good agreement with that calculated for the octahedral mixed hydroxo complex confirming our previous assumption that these complexes are formed from the MLH (MLH_2 in the case of Gly-His) by the additional coordination of the OH^-, while the amino nitrogens remain protonated (Figure 4b).

The Mössbauer spectrum of the dimethyltin(IV)−dipeptide systems detected above pH 5 can be deconvoluted in two doublets representing the $M(OH)_2$ hydrolysis product and the corresponding complex species MLH_{-1} (ML for Gly-His), respectively. According to its QS value, $M(OH)_2$ is a five-coordinated Sn(IV) compound, in which the hydroxyde ions are in axial position. The quadrupole splitting values belonging to the MLH_{-1} and ML (for Gly-His) are very close to those of the tridentate solid dimethyltin(IV)−glycylglycine complex dissolved in methanol (3.23) and in water (3.27) which has already been published.[22] This fact supports our assumption for the structure of MLH_{-1} (Gly-Gly) and of ML (Gly-His): these species are pentacoordinated complexes in which the alkyl groups and the peptide-nitrogen are in equatorial, while the carboxylate and amino donor groups are in axial position (Figure 4c). Due to the tridentate mode of coordination the structure is very distorted resulting the relatively greater difference between measured and calculated QS values.

Considering the experimental Mössbauer data (Table 4), there are no significant differences between the ML and MLH_{-1} complexes formed in dimethyltin(IV)−Gly-His system. This fact confirms that the process ML ⇔ MLH_{-1} belongs to the deprotonation of the histidine side-chain of the ligand without the metal assistance, as we assumed above on the basis of potentiometric and NMR experiments.

Conclusion

The presented results concerning the dimethyltin(IV)−Gly-Gly and −Gly-His systems, provided the first evidence that dialkyltin(IV) cations are able to promote the deprotonation of the peptide nitrogen in aqueous solution, at surprisingly low pH in fact. In this process the carboxylate is the anchoring group (assisting by chelate formation), in contrast with any other metal ion which is known to coordinate to amide nitrogen. The imidazole ring is reported to bind alkyltin(IV) cations in several proteins. However, our data do not prove the coordination of the imidazole nitrogens of the studied ligands. Consequently, if such binding occurs in some proteins, it should be forced by an intimate conformation of the imidazole side chain(s).

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