The Binding of Trialkyltin(IV) Moieties to Rat Haemoglobin, and the Structure of Model Systems, studied by Tin-119 Mössbauer Spectroscopy

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The configuration of the tin environment in binding sites of microcrystalline SnR_3–rat haemoglobin complexes (R = Me, Et, or Bu^n), as well as in model systems consisting of $Sn^{IV}R_3$ moieties in aqueous media and in organic solutions (mimicking hydrophilic and hydrophobic zones of the globin respectively) has been investigated by ^{119}Sn Mössbauer spectroscopy, through the point-charge model rationalization of the experimental nuclear quadrupole splitting, ΔE, measured at 77.3 K, and eventually by Mössbauer–Zeeman spectra taken at 4.2 K in a transverse magnetic field of 6 T. A distorted trigonal-bipyramidal (tbp) structure $SnR_3(S_{thiol})(N_{het})$ has been inferred for the haemoglobin complexes, where the ligand atoms (axial) would come from a cysteine and histidine side chain respectively, according to previous biochemical work. Moreover, the occurrence of a strong Sn–S bond (with a C–Sn–S angle consistently larger than 90°), as well as of a weak N ⟶ Sn co-ordinative interaction, has been proposed. The aqueous model systems [$Sn^{IV}Me_3$ and $Sn^{IV}Et_3$ derivatives at pH 7.40, eventually in presence of *N*-(2-hydroxyethyl)piperazine-*N*′-ethane-2-sulphonic acid (Hepes) buffer, which were reacted with cysteine] have evidenced the formation of quasi-regular ternary tbp complexes $SnR_3(S_{thiol})(N_{am})$ (the ligand atoms being axially located, coming from cysteine and Hepes respectively) where the N ⟶ Sn interaction is consistently lower than Sn–S. Slow-rate reactivities in aqueous solutions of SnR_3 cysteinates are also reported and commented upon. Essentially regular tbp species $Sn^{IV}R_3(SPh)(N_{het})$ (N_{het} from 1-methylimidazole; R = Me, Bu^n, or Ph) have been identified in benzene solution. The distortion of the tbp binding site in the haemoglobin complexes has then been assumed to be peculiar to the SnR_3–globin system. Comments based on crystalline derivatives with Sn–S bonds have been accordingly reported.

The binding of triethyltin(IV) to cat and rat haemoglobin has been widely investigated [1,2] in the context of studies on toxicity and biological activity of organotin(IV) compounds,[3,4] in order possibly to interpret on a molecular basis the observed effects. Haemoglobin tetramers bind $Sn^{IV}Et_3$ in a 1:2 mole ratio, with consistently large affinity constants.[1,2] The organometal moiety is linked to the α subunits, possibly to sulphur of cysteine 13 and imidazole nitrogen of histidine 113, the tin atom being embedded into a trigonal-bipyramidal structure with equatorial carbon and axial nitrogen and sulphur atoms.[5] The possible occurrence of facial SnC_3, as well as the co-ordination to tin by two nitrogen atoms from imidazole, have also been advanced.[1,6]

Our interest in organotin–haemoglobin (the latter taken as a model globin) systems originated from our studies on the anti-tumour activity of organotin(IV) compounds, and the related assumptions on structure–activity correlations.[7–9] The interaction of rat haemoglobin with a series of dimethyltin(IV) derivatives in aqueous solution, with formation of microcrystalline pellets, has been recently investigated by ^{119}Sn Mössbauer spectroscopy in our laboratories.[10] Aqueous model systems, consisting of the organotin reagent and sulphur and/or nitrogen ligands at pH 7.4, have also been investigated by the same technique in order to understand the reaction pathway as well as the co-ordination states within the haemoglobin 'phase'.[10] The formation of tetrahedral or trigonal-bipyramidal species, through covalent bonding to tin by S, and eventually N, donors from the globin, has been assumed to take place. On the other hand, dimethyltin(IV) derivatives where the tin environment appears as tetrahedral SnC_2S_2 would interact with the globin through hydrogen bonding and coulombic forces involving the ligand molecule.[10]

We tried also to rationalize the published ^{119}Sn Mössbauer parameters for a sample of $(SnEt_3)_2$–haemoglobin, and observed that the isomer shift could be consistent with a trigonal-bipyramidal configuration of the metal environment on the basis of the correlation with calculated partial atomic charges on tin,[11] while the point-charge model treatment of the nuclear quadrupole splitting [12] † was essentially consistent with the proposed occurrence of equatorial SnC_3 and axial S and N atoms.[13] In order possibly better to define the structural characteristics of $(SnR_3)_2$–haemoglobin systems (R = alkyl), further ^{119}Sn Mössbauer spectroscopic studies have been planned and carried out, and the results obtained are reported in the present paper. The radicals bound to the metal have been varied, and the systems $Sn^{IV}Me_3$–and $Sn^{IV}Bu^n_3$–haemoglobin have been investigated; the $Sn^{IV}Me_3$ moiety has been selected as the simplest $Sn^{IV}R_3$ species, which has been determined to bind to haemoglobin,[6] while $Sn^{IV}Bu^n_3$ would be representative of triorganotins characterized by noticeable biological activity and largely spread out into the environment.[14]

Experimental

Materials.—The compounds $SnMe_3Cl$, $SnEt_3Cl$, $SnBu^n_3Cl$, and $SnPh_3Cl$ were gifts from Schering (Bergkamen, B.R.D.) and Ciba-Geigy (Marienberg, B.R.D.); other compounds [*e.g.* $SnMe_3OH$, L-cysteine, dithizone (1,5-diphenylthiocarbazone), thiophenol, 1-methylimidazole, 8-mercaptoquinoline (Hmquin)

† In p. 484 of this ref. a misprint attributes $\eta = 0.00$ to the tbp structure A_2M–(*mer*-B_3).

hydrochloride, and Hepes = *N*-(2-hydroxyethyl)piperazine-*N'*-ethane-2-sulphonic acid] were from Ventron (Karlsruhe, B.R.D.), Fluka (Buchs, Switzerland), Sigma (S. Louis, Missouri), and Merck (Darmstadt, B.R.D.), and reagents and solvents from C. Erba (Milano, Italy).

The compounds $(SnEt_3)_2SO_4$, $SnR_3(SPh)$ (R = Me, Bu^n, or Ph), $SnPh_3(mquin)$, $SnPh_3(SCH_2CH_2NH_2)$, and $(SnPh_3)_2$-[$SC(CH_3)_2CH(NH_2)COO$], were prepared by literature methods.[15-19] A sample of $SnPh_3(mquin)$ was supplied by Dr. D. N. Kravtsov (Moscow).

Rat haemoglobin was obtained from erythrocytes of albino Wistar rats (*ca.* 250 g) according to the literature.[20]

Preparation and Analysis of SnR_3*–Rat Haemoglobin Complexes.*—Microcrystalline pellets were obtained by co-crystallization of rat haemoglobin and $Sn^{IV}Me_3$ or $Sn^{IV}Et_3$ derivatives by the procedure of Professor W. N. Aldridge (University of Surrey), described earlier.[10] The $Sn^{IV}R_3$ moiety (7 μmol), taken from $SnMe_3Cl$ (10 mmol dm^{-3}) or $(SnEt_3)_2SO_4$ (5 mmol dm^{-3}) in aqueous Hepes buffer adjusted to pH 7.40 (the latter measured with a Crison model 2002 instrument), was added to rat haemoglobin (*ca.* 3.5 μmol) in water (5 cm^3) immediately after lysing of the erythrocytes (from 5 cm^3 of rat blood), and the solution left to crystallize overnight at 4 °C. In the case of the $Sn^{IV}Bu^n_3$ derivative, a stock solution of $SnBu^n_3Cl$ (100 mmol dm^{-3}) in EtOH was employed, and the haemoglobin solution was rendered 20 mmol dm^{-3} in Hepes, pH 7.4, prior to the addition of the organometal; the amount of ethanol in the reaction mixture never exceeded 2% v/v. The synthesis of the complexes through diffusion of the organometal moieties into crystalline haemoglobin was carried out as follows. Rat haemoglobin (*ca.* 3.5 μmol) in water (5 cm^3) was added to Hepes (0.2 mol dm^{-3}, 0.5 cm^3) at pH 7.40, and left to crystallize at 4 °C for at least 1 d; the organotin derivatives (7 μmol), from the stock solutions mentioned above, were then added, the mixture gently stirred, and left at 4 °C at least overnight.

Haemoglobin was analyzed spectrophotometrically, according to the literature,[20] in the freshly prepared lysate of rat erythrocytes as well as in the pellet and supernatant after reaction with the organotin derivatives and the subsequent crystallization. The $Sn^{IV}Et_3$ and $Sn^{IV}Bu^n_3$ moieties were analyzed by the dithizone method[21,22] in their stock solutions as well as in the pellet and supernatant after reaction with haemoglobin. The method is not applicable to $Sn^{IV}Me_3$ due to partial extraction into the organic phase.

Samples of $(SnEt_3)_2$–haemoglobin were also supplied by Professor W. N. Aldridge (University of Surrey), and samples of $(SnMe_3)_2$–haemoglobin by Dr. M. Carrara and Professor U. Russo (University of Padova).

Mössbauer Spectroscopy.—The spectra were determined for absorber samples held at 77.3 K in liquid-nitrogen cryostats, the $Ca^{119}SnO_3$ (1—10 mCi, Radiochemical Centre, Amersham) sources at room temperature moving with linear velocity and constant acceleration, in a triangular waveform, using the apparatus and procedures described earlier.[10] The preparation of the absorbers (*ca.* 2 cm^3 of the reacting and model aqueous solutions, and 0.5—1.0 g of crystalline pellets of SnR_3–haemoglobin), and their quick freezing by immersion in liquid N_2 before measurement, were as previously reported.[10] The concentration of $Sn^{IV}R_3$ moieties (10 mmol dm^{-3}) in the aqueous frozen systems was selected to be of the order of that occurring in the reaction conditions (see above) and allowing the determination of reasonably well shaped Mössbauer spectra, satisfactorily fitted with Lorentzian lineshapes. The assumption of the practical correspondence of the metal environment in the frozen aqueous samples (crystalline) and in solution at room temperature, which is based mainly on the perfect reproducibility of Mössbauer parameters in replicate runs on various samples of solutions quickly frozen by immersion of the holder into liquid nitrogen, has been previously discussed.[10] Glassy absorber samples were instead obtained by the procedure above for 1 cm^3 of 0.1 mol dm^{-3} solutions of model systems in benzene, in Teflon cuvettes; for this greater concentration (yielding better resolved spectra) there was no requisite of correlation with the reaction conditions.

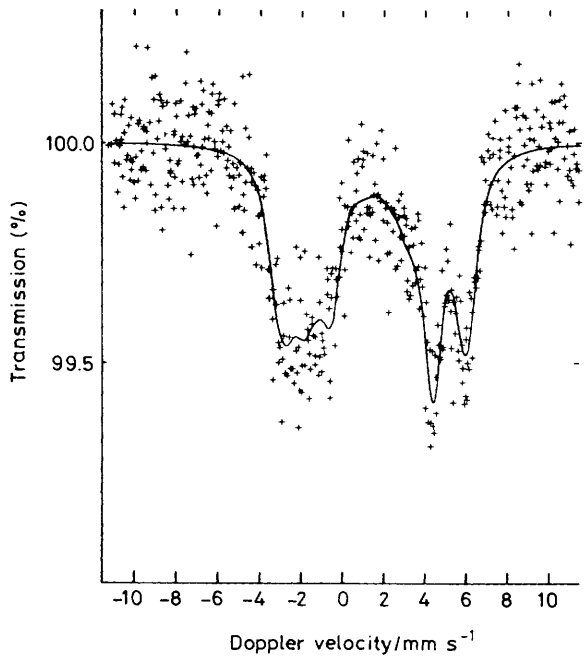


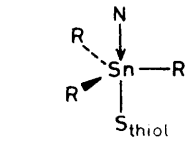
Figure 1. The Mössbauer–Zeeman spectrum of a sample of $(SnEt_3)_2$-haemoglobin: experimental data points (+) and computed spectrum (——)

The determination of the parameters δ (isomer shift), ΔE (nuclear quadrupole splitting), and Γ (full width at half height of the resonant peaks) was effected by fitting the experimental spectra with Lorentzian lineshapes.[10] The results obtained for SnR_3–haemoglobin complexes, and for model systems in water and in hydrophobic media, are reported in Tables 1—3, respectively; Table 4 reports data for model $Sn^{IV}R_3$ derivatives of thioamino acids and related species in the solid state.

The Mössbauer–Zeeman spectra[23] were recorded by the Physicochemical Measurements Unit, A.E.R.E. (Harwell), at 4.2 K in a transverse magnetic field of 6 T, and values of ΔE and η (the asymmetry parameter), reported in Table 1, were calculated by computer fitting the experimental spectra by suitable programs.

The quality of these spectra is shown in Figure 1; it is noteworthy that the measurements at liquid N_2 temperature on haemoglobin complexes, as well as on aqueous model systems with $[Sn^{IV}R_3]$ = 10 mmol dm^{-3}, yielded spectra analogous to that in Figure 1, as far as the ε°_0 values and the shape of the baseline are concerned.

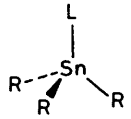
Calculations of ΔE according to the point-charge model approach[12] were carried out as usual,[10] employing partial quadrupole splitting (p.q.s.) values reported in the literature[8,10,12,24-27] and listed in the legend to Figure 2. Structures (regular) discussed in the text, in conjunction with related $\Delta E_{calc.}$ values, are reported in Figure 2. Deviations from regular geometries are accounted for by inserting the Θ and Φ angles inherent to the distortion in the usual equations for the estimate of V_{zz} (the diagonal components of the electric field gradient tensor),[28] and employing the p.q.s. values mentioned above.



(I) N = N_{het}, R = alkyl
ΔE_{calc} -2.13

(II) N = N_{am}, R = alkyl
ΔE_{calc} -2.22

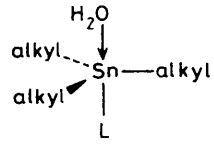
(III) N = N_{het}, R = Ph
ΔE_{calc} -1.68



(IV) L = OH, R = alkyl
$\Delta E_{calc.}$ -1.94

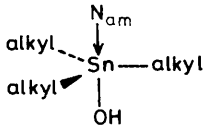
(V) L = S_{thiol}, R = alkyl
ΔE (-)1.75; 1.80*

(VI) L = S_{thiol}, R = Ph
ΔE_{calc} -1.54

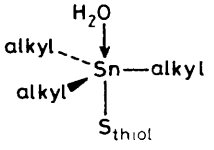


(VII) L = H_2O
ΔE (-)4.10*

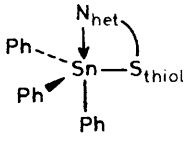
(VIII) L = OH
ΔE_{calc} -3.49



(IX)
$\Delta E_{calc.}$ -3.15



(X)
$\Delta E_{calc.}$ -2.56



(XI)
$\Delta E_{calc.}$ 0.968
η 0.535

Figure 2. The regular tetrahedral and trigonal-bipyramidal structures (idealized) discussed in the text (alkyl = Me, Et, or Bu^n), and the related point-charge model estimates of the Mössbauer nuclear quadrupole splitting parameters ΔE. The value $\eta = 0$ for the asymmetry parameter $[\eta = (V_{xx} - V_{yy})/V_{zz}]$ holds for structures (**I**) – (**X**). The following p.q.s. values, mm s^{-1}, have been employed in the calculations. Tetrahedral configurations: alkyl, −1.37; Ph, −1.26; OH, −0.40; S_{thiol}, −0.49. Trigonal bipyramidal axial: N_{am}, 0.01; N_{het}, −0.035; S_{thiol}, −0.595; H_2O, 0.18; OH, −0.13; Ph, −0.89. Trigonal bipyramidal equatorial: alkyl, −1.13; Ph, −0.98; S_{thiol}, −0.60. Symbols: N_{het}, N_{am} denote heterocyclic and amino nitrogen respectively; S_{thiol} is cysteine sulphur. * Experimental values, estimators of partial quadrupole splittings of S_{thiol} (tetrahedral)[25] and H_2O (trigonal bipyramidal, axial),[26] respectively

Results and Discussion

Structure of $Sn^{IV}R_3$ *Haemoglobin from Mössbauer Spectroscopy of Microcrystalline Pellets.*—The general correspondence of the magnitude of the nuclear quadrupole splittings, ΔE in Table 1, as well as the narrowness of the linewidths Γ, indicates the general occurrence of a single co-ordination site for the tin atoms, which is apparent in all complexes irrespective of the alkyl radicals bound to the metal, of the method of obtainment of the pellets, as well as of the type of haemoglobin (cat or rat). The assumption of the surface location of the tin binding site in haemoglobin (see Introduction) would then be further supported.

The Mössbauer–Zeeman data points (see Figure 1) were fitted by computed spectra characterized by the negative sign for ΔE and by the zero value for η (Table 1). In the context of the actual knowledge in this field, as reported in the Introduction, the latter data, in conjunction with point-charge model calculations (Figure 2), strongly support a trigonal bipyramidal (tbp) structure of type (**I**), Figure 2 (possibly distorted, as discussed in the following) or the tetrahedral structure (**V**), Figure 2, for the $(SnEt_3)_2$–haemoglobin complex in the crystalline state. The possible (in principle) occurrence of tbp isomers with facial and with meridional SnC_3 configurations,[13] which show $\eta \neq 0.00$,[12] is then definitely ruled out, according to previous assumptions based on point-charge model calculations.[13] The findings above would be reasonably extended to all complexes listed in Table 1.

A choice between the idealized regular structures (**I**) and (**V**), Figure 2, may now be effected through the absolute values of $\Delta E_{exp.,\,calc.}$. From the data in Table 1 and Figure 2, and recalling the maximum accepted difference = ±0.4 mm s^{-1} between $\Delta E_{exp.,\,calc.}$,[24] structure (**I**) would be ruled out for $(SnMe_3)$–haemoglobin, while structure (**V**) would be consistent with all $Sn^{IV}R_3$ derivatives and consequently preferred. It seems worthy to recall that the latter configuration has been taken into account for some organotin–haemoglobin complexes in solution.[1,29]

The possible occurrence of distorted structures must now be considered, in view of the complexity of the systems under study. The experimental value ΔE *ca.* 1.8 mm s^{-1}, Table 1, is for example, reproduced in the point-charge model approach by distorting the angles CSnS of (**I**), Figure 2, to *ca.* 100°. The structure of the tin sites in our microcrystalline complexes would then be in some way intermediate between (**I**) and (**V**), Figure 2, being characterized by a weak N ⟶ Sn axial interaction. In our opinion, this distorted tbp structure is the assumption most reliable, being in accordance with both the majority of the results obtained from biochemical investigations (see Introduction) and with the present Mössbauer spectroscopic data.

The Model Aqueous Systems.—These concern the $Sn^{IV}Me_3$ and $Sn^{IV}Et_3$ moieties, which are treated with haemoglobin in aqueous solution (see Experimental section).

Trialkyltin cations are likely to be bound by two molecules of H_2O to form tbp species (**VII**), Figure 2, and the methyl and butyl derivatives show ΔE = 4.10 and 4.20 mm s^{-1}, respectively, in the solid state.[26,30] Such complex cations are possibly present in aqueous solutions of $Sn^{IV}Me_3$ at acid pH,[31] consistent with ΔE_{av} of $SnMe_3Cl$ (pH 3.10—3.70), Table 2. Hydrolysis yields $SnMe_3(OH)$, with H_2O molecules possibly co-ordinated to the metal centre, and these species predominate at pH >8.0 in aqueous 3 mol dm^{-3} $NaClO_4$ at 25 °C.[32] The Mössbauer data of $SnMe_3Cl$ (pH 7—9.63) (**10**), Table 2, suggest that a single tin site is present in aqueous solution at pH > 7.0, based on the constancy of ΔE with respect to changes of pH and the narrowness of the parameters Γ; the same is likely also for $(Sn^{IV}Et_3)_2SO_4$ (**11**). Assuming complete hydrolysis of $[SnR_3(OH_2)_2]^+$ in both instances, the structure of the species formed is other than regular tetrahedral (**IV**), as well as regular tbp (**VIII**), Figure 2, since the experimental ΔE values of (**10**) and (**11**) are intermediate, and inconsistent, with respect to the corresponding point-charge model estimates for (**IV**) and (**VIII**). Distortion of the HOSnC angles in (**VIII**) to 105 ° yields $\Delta E_{calc.}$ = −2.81 mm s^{-1}, which would suggest that the eventual tbp structure of the hydrolysis products, $SnR_3(OH)(OH_2)$, is anyway severely distorted, with loosely bound axial H_2O. It seems noteworthy that these hypotheses recall our assumption on the distorted tbp configuration of the tin site in SnR_3–haemoglobin systems (see above). Moreover, the assumed distortion would reflect a tendency toward the formation of a tetrahedral type $SnR_3(OH)$ species, which would be in line with the formation of tetrahedral $SnMe_2(OH)_2$[10,33] by hydrolysis of the *trans*-octahedral aqua cation $[SnMe_2(OH_2)_4]^{2+}$; the reluctance of H_2O to co-ordinate to the metal centre in the

Table 1. ^{119}Sn Mössbauer parameters of SnR_3–haemoglobin (Haem) complexes[a]

Code no.	Haemoglobin complex of:	$[Sn^{IV}R_3]$/[Haem][b]	δ[c]/mm s^{-1}	ΔE[d]/mm s^{-1}	Γ_1[e]/mm s^{-1}	Γ_2[e]/mm s^{-1}
(1)	$Sn^{IV}Me_3$[f]	2.00	1.32	1.57	0.86	0.89
(2)	$Sn^{IV}Me_3$[g]	1.90	1.25	1.53	1.03	0.70
(3)	$Sn^{IV}Et_3$[f,h]	1.95	1.43	1.78	0.83	0.97
(4)	$Sn^{IV}Et_3$[f,i]	1.83	1.43	−1.76 (η = 0.00)[j]	—	—
(5)	$Sn^{IV}Et_3$[k]	1.85	1.48	1.74	—	—
(6)	$Sn^{IV}Et_3$[g]	1.95	1.38	1.61	0.74	0.88
(7)	$Sn^{IV}Bu^n_3$[f]	0.73—1.54	1.45	1.94	0.82	0.70
(8)	$Sn^{IV}Bu^n_3$[g]	0.96—2.35	1.50	1.77	0.63	1.03

[a] Absorber samples were microcrystalline pellets (see Experimental section) held at 77.3 K unless otherwise stated. The percentage resonant effect was ε^o_0 = 0.15 – 0.50. At least two distinct samples of each complex, coming from individual syntheses, were submitted to Mössbauer spectroscopy as far as the determinations effected in our laboratory are concerned. [b] Molar ratio, or eventual ranges of values, employed in the synthesis (see Experimental section). [c] Isomer shift with respect to room temperature (r.t.) $CaSnO_3$, average values. [d] Nuclear quadrupole splitting, av. ±0.02 mm s^{-1}.[10] [e] Full width at half height of the resonant peaks (av.) at lesser and larger velocity than the spectrum centroid, respectively. [f] Obtained by co-crystallization of $Sn^{IV}R_3$ with rat haemoglobin (see Experimental section). [g] Obtained by diffusion of $Sn^{IV}R_3$ into haemoglobin crystals (see Experimental section). [h] Absorber samples were suspensions of the pellet into the supernatant, in Hepes buffer 0.2 mol dm^{-3}, pH 7.40 (see Experimental section). [i] Data from two Mössbauer–Zeeman spectra at 4.2 K, see Figure 1 and Experimental section. A further spectrum taken at 4.2 K without applied magnetic field gave δ = 1.50, ΔE = 1.72 mm s^{-1}. [j] $\eta = (V_{xx} - V_{yy})/V_{zz}$, the asymmetry parameter.[12] [k] A cat haemoglobin complex, literature data;[1] a solution of 2.7 mmol dm^{-3} cat haemoglobin containing 5.0 mmol dm^{-3} $Sn^{IV}Et_3$ and buffered at pH 7.80 by 0.09 mol dm^{-3} Tris·HCl [Tris = 2-amino-2-(hydroxymethyl)propane-1,3-diol]. From known affinity constants[20] the binding sites of cat haemoglobin should be 90% saturated with $Sn^{IV}Et_3$ and the solution will contain little free $Sn^{IV}Et_3$.

Table 2. Aqueous model systems: ^{119}Sn Mössbauer parameters of solutions frozen at 77.3 K

Code no.	Aqueous solution[a]	Species formed[b]	δ[c]/mm s^{-1}	ΔE[c]/mm s^{-1}	Γ_1[c]/mm s^{-1}	Γ_2[c]/mm s^{-1}
(9)	$SnMe_3Cl$ (pH 3.10—3.70)	$[SnMe_3(OH_2)_2]^+$	1.50	3.87	0.86	0.90
(10)	$SnMe_3Cl$ (pH 7.00—9.63)	$SnMe_3(OH)(OH_2)$	1.24	2.80	0.79	0.72
(11)	$(SnEt_3)_2SO_4$	$SnEt_3(OH)(OH_2)$	1.30	2.98	0.93	0.81
(12)	$SnMe_3Cl$ in Hepes	$SnMe_3(OH)(NR_3)$	1.34	3.31	0.84	0.81
(13)	$(SnEt_3)_2SO_4$ in Hepes	$SnEt_3(OH)(NR_3)$	1.45	3.41	0.99	0.89
(14)	$SnMe_3Cl + H_2Cys$	$SnMe_3(SR)(OH_2)$	1.37	2.35	0.97	1.12
(14′)	$SnMe_3Cl + H_2Cys$ after 94 h at r.t.	[b]	1.29	2.80	0.85	1.05
(15)	$(SnEt_3)_2SO_4 + 2H_2Cys$	$SnEt_3(SR)(OH_2)$	1.43	2.06	1.02	1.21
(15′)	$(SnEt_3)_2SO_4 + 2H_2Cys$ after 95 h at r.t.	[b]	1.29	2.88	1.64	1.21
(16)	$SnMe_3X$[d] + H_2Cys in Hepes	$SnMe_3(SR)(NR_3)$	1.34	2.44	0.89	0.80
(16′)	$SnMe_3X$[d] + H_2Cys in Hepes after 95 h at r.t.	[b]	1.31	2.69	0.98	1.01
(17)	$(SnEt_3)_2SO_4 + 2H_2Cys$ in Hepes	$SnEt_3(SR)(NR_3)$	1.47	2.59	0.92	0.95
(17′)	$(SnEt_3)_2SO_4 + 2H_2Cys$ in Hepes after 94 h at r.t.	[b]	1.45	3.11	1.17	1.19

[a] H_2Cys = L-Cysteine. The concentrations of $Sn^{IV}R_3$ and H_2Cys were 10 mmol dm^{-3}, and that of Hepes was 0.2 mol dm^{-3} adjusted to pH 7.40. Unless otherwise stated, the solutions were taken at pH 7.40 by Hepes buffer or by addition of NaOH. The Mössbauer absorbers were generally prepared by rapidly freezing in liquid N_2 samples of the solutions just after their preparation (unprimed code numbers) or after storage at *ca.* 20 °C for the time given in the Table (primed code numbers). [b] See Results and Discussion section. [c] See footnotes to Figure 1. Each value is extracted from at least two spectra measured for individual solutions. [d] X = Cl or OH.

dihydroxide is ascribed to large electron densities transferred to tin[34] by the hydroxide ligands.

The addition of excess Hepes to $SnR_3(OH)(OH_2)$ provokes the replacement of H_2O by a tertiary amino nitrogen of this buffer with formation of nearly regular tbp species, (**IX**) of Figure 2, according to the experimental ΔE values of (**12**) and (**13**), Table 2, and the corresponding point charge estimate. Single tin sites are likely to occur according to the narrowness of the Γ parameters. The possible co-ordination of Hepes to Sn has been demonstrated in an earlier study on $Sn^{IV}Me_2$ derivatives,[10] and the related findings seem to apply equally well to the systems investigated here, in the light also of recent studies on the co-ordination to $Sn^{IV}Me_3$ by nitrogen ligands (*inter alia*) in aqueous solution.[31]

The assumptions reported above for the systems (**9**)—(**13**), Table 2, hold also for solutions even stored for several months at room temperature, whose Mössbauer parameters are practically invariant.

Cysteine reacts in aqueous solution, at pH 7.4, with $SnR_3(OH)(OH_2)$ in a 1:1 mole ratio, as indicated by the drastic change of ΔE occurring from solutions (**10**), (**11**)—(**14**), (**15**), Table 2, and according to studies of stability constants in aqueous solution.[31] The magnitude of $\Delta E_{exp.}$ for (**14**) and (**15**) suggests a consistent contribution by tetrahedral species such as (**V**), Figure 2, which is mainly evident for the $Sn^{IV}Et_3$ derivative; on the other hand, the relatively large values of the Γ parameters of (**15**), Table 2, strongly suggest the occurrence of more than one tin co-ordination site. The relationship of these systems with the hydrolyzed species $SnR_3(OH)(OH_2)$ is noteworthy *i.e.* the more charge released by the sulphur donors onto the tin centres, with respect to that induced by the hydroxides, provokes a lesser Lewis acidity of Sn with a consequent lesser tendency to co-ordinate H_2O with respect to $SnR_3(OH)$. In any event, the primary obtainment of tetrahedral species (**V**) is quite illuminating of the structure of the products of the reaction of $Sn^{IV}R_3$ with haemoglobin, in the sense that the assumed

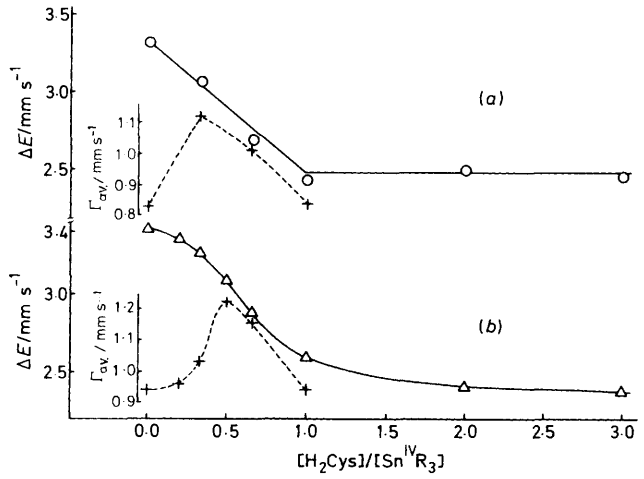


Figure 3. The 'Mössbauer titration' of (*a*) $Sn^{IV}Me_3$ or (*b*) $Sn^{IV}Et_3$ with L-cysteine (H_2Cys) in Hepes (0.2 mol dm^{-3}) at pH 7.40: trends shown by nuclear quadrupole splittings, ΔE, and full width at half height of the resonant peaks, Γ_{av}. The concentration of the $Sn^{IV}R_3$ moieties was constantly 10 mol dm^{-3}

distorted tbp structure (see above) is strengthened by these results concerning the model system.

The tbp complexes $SnR_3(OH)(NR_3)$, (**12**) and (**13**) of Table 2, with structure (**IX**), Figure 2, react analogously with cysteine, yielding tbp complexes with structure (**II**), Figure 2, as suggested by the agreement of the $\Delta E_{exp.}$ values of (**16**) and (**17**), Table 2, with the related point-charge model estimate. In these contexts, single tin sites are present initially in the solution, as suggested by the values of Γ, and contrary to findings for (**14**) and (**15**) (see later).

The stoicheiometry of the reaction is 1:1, as clearly indicated by the 'Mössbauer titration' graphs reported in Figure 3, where practically constant ΔE values are obtained for $[H_2Cys]/[Sn^{IV}R_3]$ mole ratios $\geqslant 1$, in the formation of the mixed complexes SnR_3-H_2Cys-Hepes, and this reflects previous findings on the ternary complex $SnMe_2$–$(H_2Cys)_2$–Hepes as well as the related interpretations and comments.[10] The occurrence of at least two tin sites during the reaction is evidenced by the Γ widening reported in Figure 3, while single species clearly occur at the beginning as well as at the end of the reaction. It is worth noting that the shape of the curves of Figure 3, ΔE *vs.* $[H_2Cys]/[Sn^{IV}R_3]$ mole ratio, is clearly suggestive of a lesser stability constant for the S–Sn bond in $SnEt_3$–H_2Cys–Hepes with respect to that for S–Sn in $SnMe_3$–H_2Cys–Hepes, and this would be in line with the larger inductive effects of the ethyl radicals and the consequent lesser partial positive charge located on Sn of the $Sn^{IV}Et_3$ derivative.

Trialkyltin cysteinates in aqueous solution, systems (**14**)—(**17**), Table 2, are involved in slow reactions at room temperature, as indicated by the drastic increases in the parameters ΔE [(**14′**)—(**17′**), Table 2], by the large Γ values of the $Sn^{IV}Et_3$ solutions (**15′**) and (**17′**), as well as by the formation of solid products within 2—5 d (depending upon the ambient temperature). Speculating on the possible nature of the $Sn^{IV}R_3$ species formed in solution, it could be argued that complexes (**14**) and (**15**) would continue gradually to undergo a steady aquation of the tin centre (see above) in (**V**) Figure 2, acquiring the tbp structure (**X**). In the meantime, processes of desulphuration could take place for (**14**)—(**17**), and could yield tbp species such as (**VIII**) and (**IX**). These assumptions are in line with the experimental ΔE data in Table 2 and $\Delta E_{calc.}$ of Figure 2. Analogous experimental behaviour has been detected to occur for $SnMe_3(SCH_2CH_2SO_3Na)$ in aqueous solution.[35]

The desulphuration processes could be ascribed to the gradual oxidation of free cysteine to the sparingly soluble cystine.[36] Moreover, the solid products could also consist of trialkyltin(IV) sulphides, according to previous preparative investigations;[37–39] on the other hand, a tin-free solid sample was precipitated from a solution of $SnEt_3(Cys)$ at pH 7.4. In any case, it seems hard to attribute a biological significance to these processes in connection with the trialkyltin–haemoglobin systems and also in view of the very preliminary available data which have been commented upon; further work is clearly needed in this field.

From the results obtained in the present study of aqueous model systems, we suggest the following conclusions: (*i*) the pathway of the reaction between haemoglobin and $Sn^{IV}R_3$ (R = Me or Et) in aqueous solution at pH 7.4 could merely consist of an acid–base interaction, involving a cysteine side chain of the globin and complexes such as $SnR_3(OH)(OH_2)$ and $SnR_3(OH)(NR'_3)$; (*ii*) the structure of the tin site in the SnR_3–haemoglobin complexes, *i.e.* distorted tbp possibly intermediate between (**I**) and (**V**) (see above), is certainly different from the quasi-regular tbp configuration (**II**) possibly occurring in the aqueous $Sn^{IV}R_3$–H_2Cys–Hepes systems.

$SnBu^n_3Cl$ *in Organic and Aqueous Media, and Model Systems in Organic Solvents.*—Under the conditions of the reaction between haemoglobin and $SnBu^n_3Cl$, described in the Experimental section, the formal concentration of the organometal derivative exceeds its solubility.[14] Stock solutions of $SnBu^n_3Cl$ 100 mmol dm^{-3} in ethanol give $\Delta E_{av} = 3.43$ mm s^{-1}, which suggests the occurrence of tbp species with axial Cl and EtOH ($\Delta E_{calc.} = -3.67$ mm s^{-1}). The formation of suspensions occurs in the corresponding model aqueous systems, possibly due to $SnBu^n_3(OH)$, which shows $\Delta E = 2.99$ mm s^{-1} in the solid state;[40] its formation in the present context would be consistent with studies on hydrolysis in mixed solvents.[41] The formation of tin–sulphur bonds could be assumed to take place through the reaction of $SnBu^n_3(OH)$ with cysteine, in consonance with earlier findings on homocysteinate- and ethylcysteinate-$SnBu^n_3$ systems.[38,39]

These observations would indicate the interaction of $Sn^{IV}Bu^n_3$ with the thiol groups of the cysteine side chains in haemoglobin, under the experimental conditions employed in this research. The related structural model systems, concerning hydrophobic triorganotin(IV) moieties (due to the nature of the organic radicals bound to the metal), may consist of solutions of organometal complexes in solvents such as benzene, which would mimic hydrophobic regions of the globin. A series of triorganotin(IV) thiophenolates has been selected here for this purpose (including $Sn^{IV}Me_3$ and $Sn^{IV}Ph_3$ for comparison with $Sn^{IV}Bu^n_3$), and their Mössbauer spectra in frozen solutions in benzene have been determined, in the presence also of 1:1 1-methylimidazole (mim) mimicking the eventual co-ordination by histidine side chains of haemoglobin (see Introduction). The complex $SnPh_3$(tquin) was also investigated in order to determine the order of spectroscopic response due to facial-SnC_3 tbp type configuration. Other pertinent systems reported in the literature were also taken into account. Relevant data are listed in Table 3, from which it clearly appears that the tetrahedral species $SnR_3(SPh)$ [(**18**) and (**19**) of Table 3; (**V**), Figure 2] form 1:1 complexes with mim [(**20**) and (**21**); (**I**)] which possibly correspond to the species (**22**) present in pyridine solution. Moreover, tetrahedral $SnPh_3(SPh)$ (**23**), (**VI**), is analogously co-ordinated by these nitrogen donors [(**24**) and (**25**)] into the regular tbp structure (**III**), which pertains to the complex with 4-thiopyridine (**26**) in the crystalline state;[42] the Mössbauer parameters of these tbp derivatives are drastically different from those of the *fac*-SnC_3 8-thioquinolate complexes[18] [(**27**); (**XI**)], which generally excludes the latter configuration for the tbp systems of Table 3.

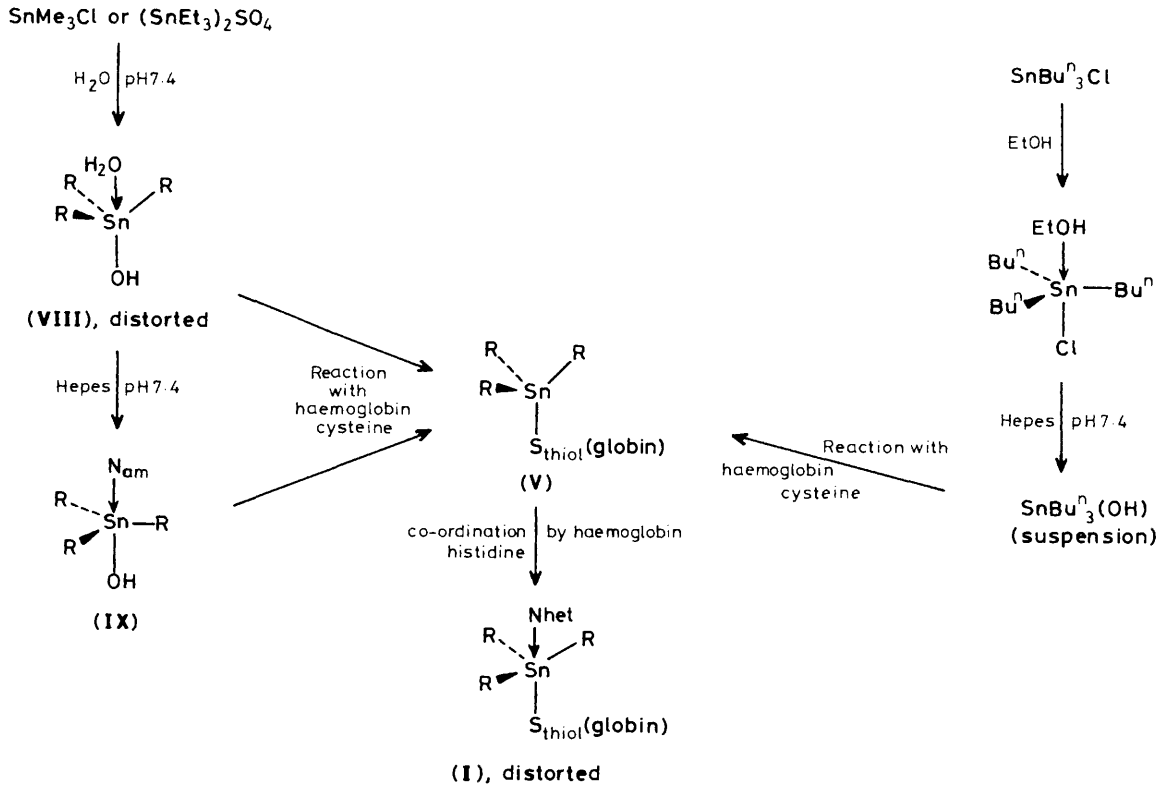


Figure 4. Schemes for the reaction of $Sn^{IV}R_3$ (R = Me, Et, or Bu^n) with haemoglobin, and possible structures of the complexes. Codes and symbols are as in Figure 2

Table 3. Model systems in hydrophobic media: ^{119}Sn Mössbauer parameters of solutions frozen at 77.3 K

Code no.	Benzene solution[a] (unless otherwise stated)	δ[b] / mm s^{-1}	ΔE[b] / mm s^{-1}	Γ_1[b] / mm s^{-1}	Γ_2[b] / mm s^{-1}
(**18**)	$SnMe_3(SPh)$	1.35	1.97	0.88	0.88
(**19**)	$SnBu^n_3(SPh)$	1.46	2.06	0.83	0.74
(**20**)	$SnMe_3(SPh)$ + mim	1.33	2.76	0.79	0.71
(**21**)	$SnBu^n_3(SPh)$ + mim	1.45	2.78	0.77	0.82
(**22**)	$SnBu^n_3(SPh)$ in py[c]	1.43[c]	2.62[c]	0.87[c]	0.88[c]
(**23**)	$SnPh_3(SPh)$	1.33	1.46	0.81	0.86
(**24**)	$SnPh_3(SPh)$ + mim	1.27	2.36	0.81	0.81
(**25**)	$SnPh_3(SPh)$ in py[c,d]	1.38[c]	2.39[c]	0.85[c]	0.85[c]
		1.52[d]	2.34[d]	—	—
(**26**)	$SnPh_3(tpy)$[d,e]	1.37[d]	2.61[d]	—	—
	neat solid	1.36[d]	2.54[d]	—	—
	pyridine solution				
(**27**)	$SnPh_3(tquin)$[f]	1.20	1.37	0.76	0.77
	neat solid[g]	1.15	1.37	0.79	0.80
	benzene solution				

[a] The solutions in benzene were 0.1 mol dm^{-3} for SnR_3L and eventually for *N*-methylimidazole (mim). [b] See footnotes to Table 1. [c] R. C. Poller and J. N. R. Ruddick, *J. Chem. Soc., Dalton Trans.*, 1972, 555; py = pyridine. [d] Ref. 43. [e] tpy = 4-thiopyridine. [f] tquin = 8-thioquinolate. [g] Average values for absorbers with thickness in the range 0.23—0.53 mg ^{119}Sn cm^{-2}.

It must be noted here that the point-charge model estimates of ΔE for the tbp structures (**I**) and (**III**), Figure 2, are consistently lower than the experimental values of the complexes (**20**)—(**22**), and (**24**)—(**26**), Table 3, to which these structures have been assigned, and the differences $\Delta E_{calc.,exp.}$ are definitely out of the correlation criteria.[24] This situation has already been discussed, *inter alia*, in connection with $SnR_3(tpy)$ complexes, where the need for smaller working values for the partial nuclear quadrupole splitting has been evidenced and justified by the large S–Sn bond distance detected in the solid, due to the possible contribution by the pyridinethione isomer.[27,42] It is evident that these discrepancies do not affect the present structural assignments, which involve a choice between the tetrahedral and tbp configurations (**V**)—(**VI**) and (**I**)—(**III**), Figure 2, respectively. Moreover, it is also clear that the tin sites in SnR_3–haemoglobin do not structurally correspond to the supposedly regular tbp model systems in benzene and pyridine of Table 3.

Solid-state Model Systems.—In addition to the models in hydrophobic media, crystalline solids could also be taken into account as model systems for SnR_3–haemoglobin derivatives where the nature of the radicals bound to the metal involves interactions with water-free zones of the globin. In this context the best model available is $SnEt_3(tpy)$, ΔE = 3.05 mm s^{-1},[43] which supposedly has the regular tbp structure (**I**), Figure 2, analogous to $Sn^{IV}Ph_3(tpy)$.[42] Moreover, the 1:1 complex of $Sn^{IV}Me_3$ with methyl *N*-benzoyl-L-histidyl-L-cysteine, ΔE = 3.35 mm s^{-1}, has also been proposed for this purpose.[44] These ΔE values are even larger than those of the tbp models in hydrophobic media (Table 3), and analogously inconsistent with the point-charge model estimate for (**I**), Table 2; these data again strongly suggest that the tin site in SnR_3–haemoglobin is largely different from the regular tbp structure (**I**).

An additional piece of structural information from solid-state models may be extracted from complexes of $Sn^{IV}R_3$ with multidentate ligands also including a thiol group. The ΔE data in Table 4, (**28**)—(**34**), clearly show that tetrahedral structures $SnR_3(SR')$, (**V**) and (**VI**), Figure 2, occur in 1:1 complexes with

Table 4. A selection of ^{119}Sn Mössbauer parameters for tetrahedral sites in triorganotin(IV) complexes with mercaptoamino acids and related compounds, in the solid state

Code no.	Compound[a]	δ[b]/mm s^{-1}	ΔE[b]/mm s^{-1}
(**28**)	$SnBu^n_3(SCH_2CO_2Na)$	1.45[c]	1.59[c]
(**29**)	$SnPh_3(SCH_2CH_2NH_2)$	1.19[d,e]	1.21[d,e]
(**30**)	$SnR_3[S(CH_2)_nCH(NH_2)CO_2H]$ } n = 1 or 2	1.39[f]	1.67[f]
(**31**)	$SnPh_3[S(CH_2)_nCH(NH_2)CO_2H]$ }	1.26[f]	1.41[f]
(**32**)	$(SnBu^n_3)_2[SCH_2CH(NHCOCH_3)CO_2]$ (inner doublet)	1.36[g,h]	1.62[g,h]
(**33**)	$(SnPh_3)_2[SC(CH_3)_2CH(NH_2)CO_2]$ (inner doublet)	1.24[e]	1.20[e]
		1.26[i]	1.31[i]
		1.20[d,j]	1.27[d,j]
(**34**)	$(SnPh_3)_2[SCH_2CH(NH_2)CO_2]$ (inner doublet)	1.35[i]	1.36[i]
(**35**)	$(SnBu^n_3)_2$ glutathione (inner doublet)	1.41[g,h]	1.76[g,h]
(**36**)	$(SnPh_3)_2$ glutathione (inner doublet)[k]	1.22[h,k]	1.42[h,k]

[a] Glutathione is the tripeptide γ-L-glutamyl-L-cysteinylglycine. [b] See footnotes to Table 1. Data refer to 77.3 K unless otherwise stated. [c] C. H. Stapfer and R. H. Herber, *J. Organomet. Chem.*, 1973, **56**, 175. [d] This work. The thicknesses of the absorbers were of the order of 0.5 mg ^{119}Sn cm^{-2}. [e] R. Barbieri, F. Di Bianca, A. Silvestri, E. Rivarola and F. Huber, Abstracts of the XV Congresso Nazionale di Chimica Inorganica, Bari, Italy, 27th September—1st October, 1982, p. 237. [f] Ref. 39. R = Butyl, cyclohexyl, or neopentyl. Data are average values for five compounds $Sn^{IV}R_3$ and two $Sn^{IV}Ph_3$, respectively. [g] Ref. 38. [h] J. D. Cashion, G. Domazetis, and B. D. James, *J. Organomet. Chem.*, 1980, **185**, 433. [i] O. A. Bamgboye, T. T. Bamgboye, and P. G. Harrison, *J. Organomet. Chem.*, 1986, **306**, 17. [j] Average data in the temperature range 77.3—120.3 K. The analogous parameters for the outer doublet (lines 1—4) are δ = 1.23 and ΔE = 2.66 mm s^{-1}; the values of slopes $d\ln A/dT = d\ln f_a/dT$ are -1.85×10^{-2} K^{-1} and -1.66×10^{-2} K^{-1} for the internal and external doublet respectively (A is the total area under the resonant peaks, f_a the recoil free fraction). Note the difference with data in (*i*) for ΔE of the outer doublet. [k] Parameters refer to the peaks 2 and 3 of the four-line spectra, and have been recalculated from published δ and ΔE data (see *h*) which refer to peaks 1 and 3, and 2 and 4, as the component doublets respectively.

mercaptoamino acids despite the basic (Lewis) carboxyl and amino groups; the latter are in turn involved in co-ordination to additional $Sn^{IV}R_3$ moieties. The same tetrahedral bonding situation also takes place in $Sn^{IV}R_3$ complexes with the tripeptide glutathione, (**35**) and (**36**). These data would again suggest the possibility of a tetrahedral type configuration of the tin environment in the SnR_3–haemoglobin complexes.

Conclusions

The results obtained from the present ^{119}Sn Mössbauer spectroscopic study may be summarized by Figure 4, for both the possible reaction pathways and the structural implications.

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