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The Interaction of Deoxyribonucleic Acid with Methyltin(IV) Moieties in Solution Studied by Small-angle X-ray Scattering, Circular Dichroism and UV Spectroscopy

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The nature of calf thymus DNA in aqueous solution, in the presence of the organotin(IV) species $Sn^{IV}Me_3$, $Sn^{IV}Me_2$ and $Sn^{IV}Me$, possibly partly hydrolysed and/or hydrated, was investigated by small-angle X-ray scattering (SAXS), ultraviolet spectroscopy (UV) at different temperatures and circular dichroism (CD). The results are compared with those of previous ^{119}Sn Mössbauer studies on condensed DNA phases. The effects of tin–phosphate oxygen bonding on the DNA melting profile in DNA–$Sn^{IV}Me$ systems are in agreement with previous reports on DNA–Main Group metal ion interactions. The structure and conformation of the DNA double helix are not influenced by $Sn^{IV}Me_n$ species, even in experimental conditions near the onset of DNA condensation. Gel electrophoresis indicates that DNA is not degraded by $Sn^{IV}Me_n$; the X-ray radii of gyration of the DNA cross-section and its mass per unit length, determined by SAXS from aqueous solutions, are constant and independent of the nature and concentration of the $Sn^{IV}Me_n$ species present in the solutions. The invariance of the interparticle distances in solution at very low ionic strengths, in the presence of increasing amounts of $Sn^{IV}Me_n$ compounds, even approaching DNA precipitation, suggests that the condensation process involves isolated DNA chains. Copyright © **2000 John Wiley & Sons, Ltd.**

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INTRODUCTION

The molecular basis of the biological activity of organotin compounds is of interest (see, for example, Ref. 1) because of their widespread use[2] and subsequent release into the environment,[3] where they interact with living organisms.[4] Their application as possible antitumor agents has also been considered (e.g. Ref. 5)

In this context, the bonding and structure of the tin environment have been investigated by ^{119}Sn Mössbauer spectroscopy[6–9] in a series of organotin–deoxyribonucleic acid (DNA) condensates obtained by addition of organotin halides in ethanol solutions to aqueous DNA. DNA condensation is mainly due to electrostatic interactions between $Sn^{IV}R_n$ moieties (n = 1–3) and the phosphodiester groups of DNA.[6–10] SnR_2(DNA phosphodiester)$_2$ (R = Me, Et) and $SnEt_3$(DNA phosphodiester) stoichiometries have been determined in lyophilized condensates by variable-temperature ^{119}Sn Mössbauer spectroscopy.[8] The existence of octa-

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hedral $SnR_2(H_2O)_2[O_2P(OR')_2]$, and trigonal bipyramidal $SnR_3(H_2O)[O_2P(OR')_2]$ tin environments, have been established by point-charge modelling of the ^{119}Sn Mössbauer nuclear quadrupole splittings.[7,8] These findings indicate the existence of solid-state complexes of $Sn^{IV}R_2$ and $Sn^{IV}R_3$ moieties with mononucleotides and phenylphosphates.[11,12] Moreover, ^{119}Sn Mössbauer studies in aqueous solutions at neutral pH (frozen to 77.3 K) indicate that $SnMe_2(OH)_2$ and $SnMe_3(OH)$ are coordinated by phosphate and D-ribose 5-phosphate,[13] while no interactions occur with adenosine 3′:5′-cyclic monophosphate, dimethylphosphinate and DNA.[13] Potentiometric studies in aqueous solutions ($pH \leq 6.5$) gave evidence for the formation of 1:1 complexes between $SnMe_3(H_2O)_2^+$ and adenosine- or inosine-5′-monophosphates through phosphate oxygens.[14]

The structure and polyelectrolyte properties of DNA in aqueous solution have been previously investigated by SAXS, as a function of ionic strength, DNA concentration and temperature.[15,16] The structural parameters, radius of gyration of the cross-section and mass per unit length give evidence of the diameter of the DNA chains, which behave as long stiff rods, independently of ionic strength, DNA concentration and biological source. It is also possible to obtain information on the interparticle distances, from the interference peak present in the scattering pattern of native DNA solutions at very low ionic strength.

Circular dichroism (CD) and UV absorption spectroscopies are widely employed in the structural characterization of nucleic acids in solutions.[17–19] Thermal denaturation of DNA, and the melting temperature, T_m, as determined by variable-temperature UV spectroscopy, give indications on binding sites of metals and organometallic derivatives in nucleic acids: binding to nitrogen bases destabilizes the double helix and decreases T_m, while the reverse occurs in the case of binding to phosphodiester groups.[20–23] The effect of metal binding on the UV and CD spectra of nucleic acids, poly- and mono-nucleotides and nucleosides, has been reviewed recently.[24]

We report results of an extension of these studies to representative $Sn^{IV}Me_3$, $Sn^{IV}Me_2$ and $Sn^{IV}Me$ systems interacting with DNA in aqueous solution using small-angle X-ray scattering (SAXS), circular dichroism and variable-temperature UV spectroscopy, in order to investigate the influence of methyltin(IV) chlorides on the secondary structure of DNA in its native form. In particular, the aim of the following investigations is to establish whether the binding mode of organotin(IV) to DNA, detected in the condensed phase, also occurs in aqueous solutions. For this purpose, the experimental techniques applied here are among the most efficient to establish the preferential interaction of metal derivatives with the inner or outer region of DNA, because they give complementary information. In fact, eventual interaction of cationic species with the phosphate groups should leave the secondary structure and hence its chirality and cross-section diameter of DNA almost unchanged; at the same time the melting temperature should increase, because of the stabilization of the electrostatic repulsion between the negatively charged phosphate groups. In contrast, the interaction with the nitrogen bases should produce a change in DNA helicity, a decrease in the diameter of the cross-section due to the axial elongation of the double helix, detectable by changes in the CD spectrum and possibly in the SAXS data, and a destabilization of the secondary structure accompanied by a decrease of the melting temperature. Moreover, information about the interparticle distances obtained by SAXS gives insight into the mechanism of DNA condensation by means of the organotin(IV) compounds.

EXPERIMENTAL

Materials and methods

Calf thymus DNA, $M \approx 10^6$, was from Serva Feinbiochemica (Heidelberg, Germany); $SnMeCl_3$, $SnMe_2Cl_2$ and $SnMe_3Cl$ were from Aldrich Italia (Milan, Italy) and Tris–HCl was from Sigma (St. Louis, MO, USA); other reagents and solvents were from C. Erba (Milan, Italy). The purity of the commercial organotin(IV) compounds was checked by their melting points; $SnMe_2Cl_2$ and $SnMe_3Cl$ were recrystallized from benzene and light petroleum respectively.[8,9,24]

DNA was resuspended in 1 mmol dm^{-3} tris-(hydroxymethyl)aminomethane (Tris), pH 7.5, and dialysed, for at least six hours, against a solution containing 5 mmol dm^{-3} ethylenediamine-*N*,*N*,*N*′,*N*′-tetra-acetate disodium salt (Na_2H_2 EDTA) and the appropriate buffer, at pH 7.5, and five changes of the appropriate buffer. The dialysis bag (Visking, Prodotti Gianni, Milan, Italy; *M* cutoff 12000–14000 Da) had been treated previously following standard procedures.[25] All DNA

solutions were extensively degassed under vacuum before the measurements were taken.

The concentrations of the aqueous stock solutions of DNA were around 19 mmol dm^{-3} (monomer units), as determined by UV spectrophotometry, in appropriate dilute samples, with a Kontron Uvikon 922 (Neufahrn, Germany) or a Varian Cary 1E (Milan, Italy) instrument, using a value of 7000 $dm^3\ mol^{-1}\ cm^{-1}$ for the molar absorptivity, ε, at 260 nm.[26] The concentration was expressed in mg cm^{-3} assuming a molecular weight of 315 Da per monomer.[8] The pH of the solutions or supernatants was measured with Radiometer PHM62 (Copenhagen, Denmark) or Crison (Barcelona, Spain) pH meters equipped with a glass electrode.

Small-angle X-ray scattering

SAXS experiments were performed on calf thymus DNA solutions of about 5 mg cm^{-3} (monomer units), in the presence of mono-, di- and trimethyltin(IV) moieties, added as chloride salts from 0.05 mol dm^{-3} aqueous acidic solutions in 1 mmol dm^{-3} Tris–HCl, in various ratios r = [Sn]/[$DNA_{phosphate}$] (mmol $Sn^{IV}Me_n$/mmol DNA monomer) and in three different conditions: 1 mmol dm^{-3} Tris–HCl, 1 mmol dm^{-3} Tris–HCl 150 mmol dm^{-3} NaCl and 150 mmol dm^{-3} Tris–HCl.

The X-ray scattering experiments were performed on the double-focusing monochromator-mirror camera X33[27] of the EMBL in HASYLAB on the storage ring DORIS III of the Deutsches Electronen Synchrotron (DESY) at Hamburg, using a linear position-sensitive gas detector with delay line readout[28] with standard data acquisition and evaluation systems.[29] The observation range, using a 2 m camera length, was $0.01\ nm^{-1} \leq s \leq 0.4\ nm^{-1}$, where $s = 2\sin\theta/\lambda$, 2θ is the scattering angle and $\lambda = 0.15$ nm is the wavelength of the incident radiation.

The scattering patterns of the solutions and corresponding solvents were recorded in ten separate 1 min time frames, to monitor radiation damage. The solvent scattering was subtracted, then the data were normalized to the intensity of the primary beam and corrected for the detector response following standard procedures[30] implemented in the program SAPOKO (D. Svergun, M.H.J. Koch, unpublished results). For rodlike molecules the linear extrapolation of a fit of $\log(sI(s))$ vs s^2 to $s = 0$ yields a slope corresponding to the radius of gyration of the cross-section of the rod, and an intercept proportional to the mass per unit length. The extrapolation was made in a range where interference effects were negligible and the relation $4\pi^2 s^2 R_c^{\ 2} \leq 1$ is valid,[31] using the programs OTOKO[29] and GNOM.[32,33]

Gel electrophoresis

Agarose (1%) gel electrophoresis of DNA samples used for SAXS experiments was carried out in 40 mmol dm^{-3} Tris–acetate buffer 1 mmol dm^{-3} EDTA, pH 8, using standard procedures.[34] *Eco*RI plus *Hin*dIII restriction fragments of phage λ DNA (Sigma, St Louis, MO, USA) were used as molecular-weight standards.

Circular dichroism and variable-temperature UV spectroscopy

CD and variable-temperature UV were performed on calf thymus DNA solutions in 1 mmol dm^{-3} or 10 mmol dm^{-3} Tris–HCl. The final DNA monomer concentration was 0.02 mg cm^{-3} or 0.31 mg cm^{-3}. The spectroscopic investigations of DNA solutions in the presence of $SnMe_nCl_{4-n}$ (n = 1–3) were planned to determine the DNA–methyltin(IV) binding modes and DNA conformation, in conditions analogous to those of the previous condensed-phase studies.[6–9] Hence $SnMe_nCl_{4-n}$ (n = 1–3) were added from 0.01 or 0.1 mol dm^{-3} ethanol solutions to aqueous DNA solutions at neutral pH (max. 3.2% ethanol in the final solution). The [Sn]/[$DNA_{phosphate}$] ratios, r, were the same as for the condensed phases and were selected on the basis of assumed $Sn^{IV}Me_n$ valences and the dependence of ^{119}Sn Mössbauer parameters on r, reported elsewhere.[6–9,13]

CD spectra, four accumulations for each spectrum, were recorded at room temperature in the range 320–200 nm on a Jasco (Tokyo, Japan) J-715 spectropolarimeter, using a 1-cm or 0.1-cm pathlength cuvette, and the buffer baseline was subtracted.

Variable-temperature UV spectra were recorded in the range 320–220 nm on a Varian Cary 1E spectrophotometer (Milan, Italy), equipped with a double-cell Peltier thermostating system (Varian, Milan, Italy), using 1- or 0.1-cm pathlength cuvettes. T_m values were determined from the inflection point of the melting profile: the hyperchromicity at 258 nm was plotted against temperature and the central part of the sigmoid fitted with a fourth-degree polynomial.

RESULTS AND DISCUSSION

The organotin(IV) species present in aqueous solutions are determined by the respective hydrolysis constants, which have been established mainly by potentiometry.[35,36] Recent results are available for $Sn^{IV}Me_3$[14,37] and $Sn^{IV}Me_2$.[38–40] The hydrolysis of $Sn^{IV}Me$ has been studied by ^1H and ^{119}Sn NMR spectroscopy[41,42] and potentiometry.[43] Briefly, from the reported results,[14,35–43] the main, possibly hydrated, species existing in aqueous solutions in different pH ranges, may be assumed to be:

(a) pH ≈ 1–5: $SnMe_3^+$; $SnMe_2^{2+}$; $[(SnMe_2)_2(OH)_2]^{2+}$; $SnMe(OH)^{2+}$; $SnMe(OH)_2^+$.
(b) pH ≈ 5–7.5: $SnMe_2(OH)^+$; $[(SnMe_2)_2(OH)_3]^+$; dimeric or polymeric $Sn^{IV}Me$ species, Sn–O–Sn bridged.
(c) pH ≈ 7.5–10.5: $SnMe_3(OH)$; $SnMe_2(OH)_2$.
(d) pH ≈ 8.5–11: $SnMe(OH)_3$.

In ethanol solutions, molar conductances of $SnMe_3Cl$ and $SnMe_2Cl_2$ suggest the existence of nearly undissociated species,[7] in line with other data on organotin(IV) chlorides in organic solvents.[44–46] The addition of organotin(IV) chlorides from 0.1 mol dm^{-3} concentrated ethanol solutions, to aqueous native DNA in 1 mmol dm^{-3} Tris–HCl at neutral pH, induces DNA condensation with the formation of gel phases, interpreted as the neutralization of the negative charge of phosphodiester groups by cationic organotins.[6–9] In the solutions at low concentrations of DNA monomer and organotin chlorides used here, organotin moieties could interact with both phosphodiester groups and H_2O. The importance of water in assisting the coordination of $Sn^{IV}Me_2$ to phosphate oxygens of DNA has recently been established.[47] The latter would result in the formation of hydrolysis products of $Sn^{IV}Me_n$.

The DNA melting profile[21,48,49] in the system DNA–$Sn^{IV}Me$ (Fig. 1) displays an increase in T_m as r increases from 0.33 to 0.66, corresponding to stabilization of the double helix possibly due to counterion coordination to the phosphodiester group.[21,49] Addition of $Sn^{IV}Me_2$ or $Sn^{IV}Me_3$ has no effect on the DNA melting profiles, in line with the reported invariance of ^{119}Sn Mössbauer parameters ΔE in aqueous $SnMe_2(OH)_2$–DNA and $SnMe_3(OH)$–DNA systems.[13] The behaviour of $Sn^{IV}Me$ (Fig. 1) may be attributed to the occurrence of cationic, hydrolysed species; in fact, neutral $SnMe(OH)_3$ is only dominant at pH ≥ 8.5, as indicated above. Analogous effects on T_m have been reported[21,50-52] for a series of DNA–Main Group metal ion systems (Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}; r = 0.6–2.5), the increase of T_m by the cations being attributed to metal ion–phosphodiester interaction.[21,50-52] The results obtained indicate that there is a stabilizing interaction between $Sn^{IV}Me$ and DNA. The same interaction cannot be inferred for $Sn^{IV}Me_2$ and $Sn^{IV}Me_3$.

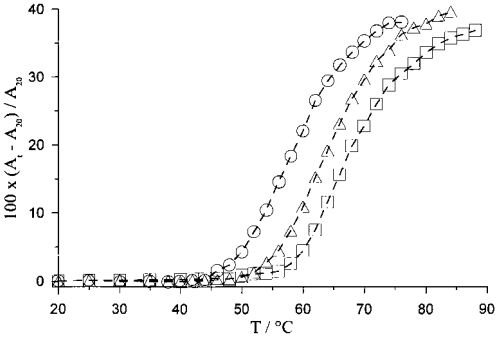

Figure 1 Melting profile of calf thymus DNA, 7.10×10^{-2} mmol dm^{-3} (monomer units) in 1 mmol dm^{-3} Tris–HCl, in the presence of $Sn^{IV}Me$ (added from $SnMeCl_3$ in ethanol, approx. 0.5% ethanol in the r = 0.66 solution; see the Experimental section.) Percentage increase of the absorbance at 258 nm vs T: ○, r = 0.00, pH = 7.5, T_m = 57 °C; △, r = 0.33, pH = 7.4, T_m = 62 °C; □, r = 0.66, pH = 7.2, T_m = 66 °C.

The ultraviolet and circular dichroism spectra of calf thymus DNA are invariant in the presence of $Sn^{IV}Me_n$ moieties at low $r = [Sn^{IV}Me_n]/$ [DNA monomer], at nearly neutral pH, as shown in Fig.2. Similar findings have been reported for DNA–Mg^{2+} ($r \leq 6$);[53] DNA–Pb^{2+} (r = 0.5–3.0);[54] DNA–Ca^{2+}, DNA–Sr^{2+}, DNA–Be^{2+} (r = 0.5)[55] and attributed to counterion binding to DNA phosphodiester[53,54] with the consequent stabilization of the double helix. In agreement with the results of the melting studies, the interaction between DNA and $Sn^{IV}Me_2$ or $Sn^{IV}Me_3$ species cannot be assumed to occur, because these would consist of the hydrolysis products described above, at neutral pH.

As far as organometallic species are concerned, $HgMe(OH)$[22,23] and $SnMe_2(OH)_2$[56] do not induce variations in the CD and UV spectra of DNA at $r \leq 1.0$, while large changes have been reported at high r values and interpreted in terms of DNA denaturation due to binding of $Hg^{II}Me$ to DNA nitrogen bases[22,23] as well as to DNA conformational transitions induced by $Sn^{IV}Me_2$.[56] The results are in agreement with the trends detected in DNA solutions in the presence of a large excess of Ca^{2+},[57] or other Main Group metal ions. $SnMe_2(OH)_2$ species can be assumed to interact

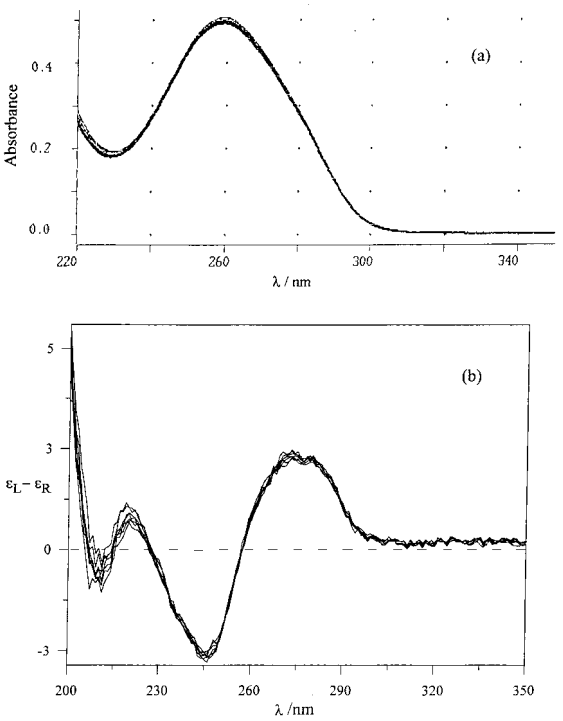

Figure 2 Examples of UV (a) and CD (b) spectra of calf thymus DNA and DNA–$Sn^{IV}Me_n$ systems. [DNA monomer] = 7.10 10^{-2} mmol dm^{-3} in Tris–HCl (1 mmol dm^{-3}); $Sn^{IV}Me_n$ = $Sn^{IV}Me$, added from ethanol solutions of $SnMeCl_3$ (see Experimental); r = moles $Sn^{IV}Me$ per mol of DNA monomer = 0.00–1.00; pH = 7.3–7.5. Analogous spectra are obtained for DNA–$Sn^{IV}Me_3$ and DNA–$Sn^{IV}Me_2$ systems r = 0.00–0.10, pH = 7.3–7.5, as well as for DNA–$Sn^{IV}Me_n$, n = 1–3, pH = 6.1–7.1 (n = 3, r = 1–5; n = 2, r = 0.5–1.0; n = 1, r = 0.33–0.66).

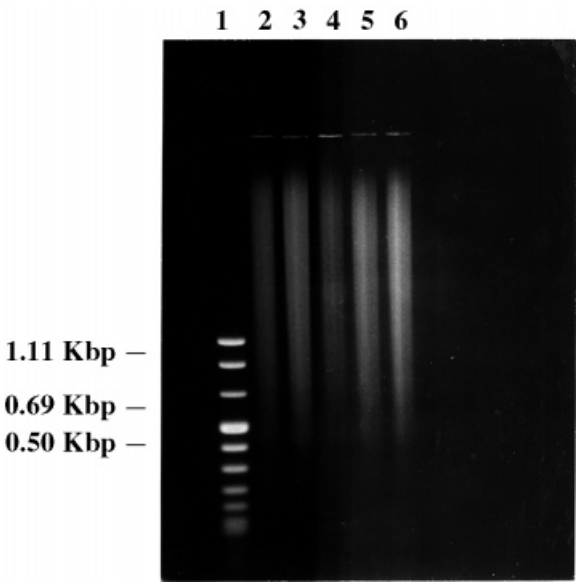

Figure 3 Agarose electrophoresis gel of DNA. Lane 1: *Eco*I plus *Hind*III restriction fragments of Phage λ DNA. Lane 2: calf thymus DNA, 6 mg cm^{-3} (in monomer units), in 1 mmol dm^{-3} Tris–HCl, pH = 7.0. Lanes 3– 6: electrophoresis of representative $Sn^{IV}Me_n$ (n = 1–3)-calf thymus DNA (2–5 mg cm^{-3} in monomer units) systems, in 1 mmol dm^{-3} Tris– HCl aqueous solutions. For the hydrolysed $Sn^{IV}Me_n$ species present at different pH values, see the Results and Discussion section. r = mmol $Sn^{IV}Me_n$ /mmol DNA monomer. Lane 3: $Sn^{IV}Me_3$, r = 1.0, pH = 5.0. Lane 4: as in lane 3, r = 0.75. Lane 5: $Sn^{IV}Me_2$, r = 0.25, pH = 6.0. Lane 6: $Sn^{IV}Me$, r = 1.0, pH = 6.0.

with both nitrogen bases and phosphodiester groups, given the T_m increase from r = 1 to r = 2, and r = 20 to r = 50, and the decrease from r = 5 to r = 10–15.[57] To our knowledge, such T_m trends[56] have not been reported previously for DNA–metal derivative systems.

Gel electrophoresis of organotin(IV)–DNA solutions indicates that DNA is not degraded (see for example, Fig. 3). All systems investigated here thus consist of unaltered native calf thymus DNA, and organotin(IV) species, in essentially aqueous solutions, since the final ethanol volume concentration is less than 3%.

The log($sI(s)$) vs s^2 plots of 5 mg cm^{-3} calf thymus DNA solutions in three different conditions: 1 mmol dm^{-3} Tris–HCl, 1 mmol dm^{-3} Tris–HCl/150 mmol dm^{-3} NaCl and 150 mmol dm^{-3} Tris–HCl, are shown in Fig. 4. At the lowest ionic strength an interference peak is visible around s = 0.05 nm^{-1}, which vanishes at higher ionic strength. This peak is characteristic of short-range order in solution, with an interparticle distance of about 20 nm, due to electrostatic repulsion of the negatively charged DNA rods.[15]

Addition of organotin(IV) compounds markedly decreases the pH of the solution and the solubility of DNA. The pH values and the radii of gyration of the cross-section of DNA, obtained from SAXS of 5 mg cm^{-3} DNA in 1 mmol dm^{-3} Tris–HCl (Fig. 5), or in 150 mmol dm^{-3} Tris–HCl, or in 1 mmol dm^{-3} Tris–HCl/150 mmol dm^{-3} NaCl solutions, and in the presence of methyltin(IV) compounds, are reported in Table 1.

Similar R_c data were measured for a series of systems DNA calf thymus–$Sn^{IV}Me_n$, in Tris–HCl 1 mmol dm^{-3}, other experimental conditions being as follows:

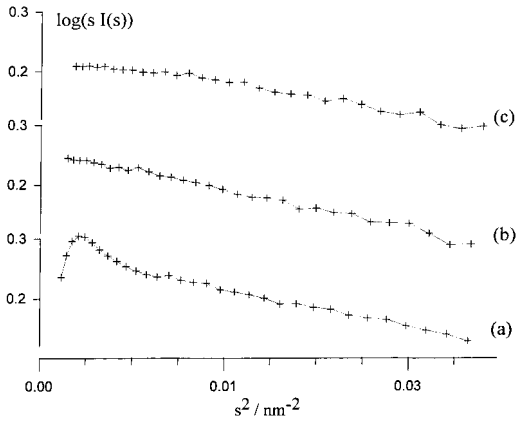

Figure 4 Variation of the SAXS intensity (arbitrary units) $\log(sI(s))$ with s^2 for calf thymus DNA (5 mg cm^{-3} in monomer units) in Tris–HCl aqueous solutions, pH 7.0, at concentrations of (a) 1 mmol dm^{-3}; (b) 150 mmol dm^{-3}; (c) 1 mmol dm^{-3} in presence of 150 mmol dm^{-3} NaCl. The curves have been displaced along the ordinate for better visualization.

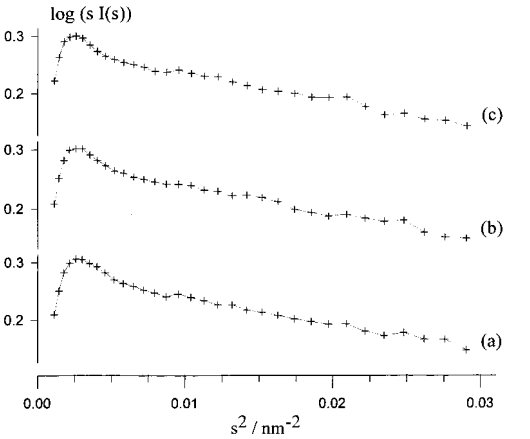

Figure 5 Variation of the SAXS intensity $\log(sI(s))$ (arbitrary units) with s^2 of calf thymus DNA (5 mg cm^{-3} in monomer units) in $Sn^{IV}Me_n$ (n = 1–3)–DNA systems in 1 mmol dm^{-3} Tris–HCl aqueous solutions. For the hydrolysed $Sn^{IV}Me_n$ species present at different pH values, see the Results and Discussion section. r = mmol $SnMe_n$/mmol DNA monomer. (a) $Sn^{IV}Me_3$, r = 0.10, pH 6.3; (b) $Sn^{IV}Me_2$, r = 0.05, pH 6.0; (c) $Sn^{IV}Me$, r = 0.01, pH 6.6. The curves have been displaced along the ordinate for better visualization. The patterns are identical at lower pH values: curves (b) and (c) are identical to those obtained at pH 4.8 and 5.4 respectively.

(1) $SnMe_3^+$; $SnMe_3(OH)$ (r = 0.01–0.50; pH 5.1–6.9; [NaCl] = 0.0 or 150.0 mmol dm^{-3}): R_c = 0.97–1.03 nm.
(2) $SnMe_2^{2+}$; $SnMe_2(OH)^+$; $[(SnMe_2)_2(OH)_2]^{2+}$; $[(SnMe_2)_2(OH)_3]^+$ (r = 0.05–0.25; pH 4.2–5.4; [NaCl] = 0.0 or 150.0 mmol dm^{-3}): R_c = 0.91–1.00 nm.
(3) $(SnMe)_n$, Sn–O–Sn bridges (r = 0.05; pH 5.4; [NaCl] = 0.0 mmol dm^{-3}): R_c = 1.11 ± 0.04 nm.

DNA condensates are formed in analogous systems containing $SnMe(OH)^{2+}$ and $SnMe(OH)_2^+$, r = 0.05–0.25, pH 3.9–4.9, [NaCl] = 0.0 or 150.0 mmol dm^{-3}.

Acid solutions originate from hydrolysis of organotin(IV) moieties, forming the species listed in Table 1. DNA condensation is induced by the cationic organotin(IV) species (see Table 1), in line with the results of previous studies on DNA condensates induced by $SnMe_3^+$ and $SnMe_2^{2+}$ at pH ≈ 2.5–3.5,[6,7] due to electrostatic interactions with DNA phosphodiester.[6–9]

No variations of the SAXS features of DNA were observed upon addition of methyltin(IV) compounds in either 150 mmol dm^{-3} Tris–HCl or 1 mmol dm^{-3} Tris–HCl/150 mmol dm^{-3} NaCl (Table 1). At these salt concentrations, no interparticle interference is detectable, even at lower pH values (Table 1).

In 1 mmol dm^{-3} Tris–HCl, at very low ionic strength, the interference maximum of DNA decreases upon addition of larger amounts of $Sn^{IV}Me_3$ and $Sn^{IV}Me_2$ and at lower pH values (Fig. 5); this is analogous to the effect of increasing the ionic strength. When $Sn^{IV}Me$ is added at ratios r above 0.01, macroscopic condensation of DNA and precipitation occur.

In the case of $Sn^{IV}Me_3$ and $Sn^{IV}Me_2$, the radii of gyration of the cross-section and the mass per unit length are independent, within experimental error, of the ionic strength, pH and r values, indicating that the structure of DNA is conserved.

Increasing the amount of methyltin(IV) in solution, the position of the interference maximum remains fixed around s = 0.05 nm^{-1} (Fig. 5), even in conditions of incipient precipitation. This suggests that the interparticle DNA distances are fixed at about 20 nm and that the condensation process involves isolated DNA particles, rather than intermolecular DNA aggregates.

CONCLUSIONS

The behaviour of nucleic acid–di- and tri-methyl-

Table 1 Radii of gyration, R_c, of the cross-section and extrapolated intensity at zero angle, $I(0)$, of native calf thymus DNA[a] in aqueous solutions, in the presence of $Sn^{IV}Me_n$ species (n = 1–3), as determined by SAXS

Tri-, di- and mono-methyltin(IV) species[b]	r[c]	pH[d]	Tris–HCl[d] (mmol dm^{-3})	R_c (nm)	$I(0)$[e]
—[d,f]	0.00	7.0	1.0	1.00 ± 0.03	12.2 ± 1.8
—[f]	0.00	8.1	150.0	0.97 ± 0.04	10.9 ± 2.8
$SnMe_3^+$; $SnMe_3(OH)$	0.05	6.6	1.0	0.98 ± 0.04	11.6 ± 2.7
$SnMe_3^+$; $SnMe_3(OH)$	0.10	6.3	1.0	0.99 ± 0.04	12.4 ± 2.6
$SnMe_3(OH)$	0.10	8.1	150.0	0.96 ± 0.04	10.0 ± 3.5
$SnMe_3(OH)$	0.80	7.9	150.0	0.94 ± 0.05	9.5 ± 2.4
$SnMe_2(OH)^+$; $[(SnMe_2)_2(OH)_3]^+$	0.01	6.7	1.0	0.99 ± 0.04	11.6 ± 2.2
$SnMe_2(OH)^+$; $[(SnMe_2)_2(OH)_3]^+$	0.05	6.0	1.0	0.97 ± 0.05	12.1 ± 2.7
$SnMe_2(OH)_2$	0.10	8.0	150.0	0.94 ± 0.03	10.3 ± 2.9
$SnMe_2(OH)_2$	0.70	7.7	150.0	0.94 ± 0.04	10.9 ± 2.6
$(SnMe)_n$, Sn–O–Sn bridges	0.01	6.6	1.0	0.98 ± 0.04	11.9 ± 3.4
$(SnMe)_n$, Sn–O–Sn bridges	0.70	7.4	150.0	1.05 ± 0.05	15.9 ± 2.3
$SnMe(OH)_3$; $(SnMe)_n$, Sn–O–Sn bridges	0.10	8.0	150.0	0.94 ± 0.04	10.6 ± 3.7

[a] Calf thymus DNA: 5 mg cm^{-3} in monomeric units.
[b] Dominant species, as estimated by $Sn^{IV}Me_n$ hydrolysis constants (see Results and Discussion section) at the indicated solution pH, hydrated species being formed.
[c] $Sn^{IV}Me_n$/DNA phosphate (mol/mol).
[d] DNA solutions (without added $Sn^{IV}Me_n$).
[e] I(0) (relative units) is proportional to the mass per unit length of the DNA rod; see the Experimental section.
[f] In the presence of NaCl (150.0 mmol dm^{-3}): R_c = 0.99 ± 0.05 nm.

tin(IV) systems, in aqueous solutions, investigated in the context of the present work from the standpoint of nucleic acid structure, is different from that of $Sn^{IV}Alk_2$–and $Sn^{IV}Alk_3$–DNA condensates, studied by ^{119}Sn Mössbauer spectroscopy.[6–9] Melting-temperature data give evidence for tin bonding by DNA phosphodiester groups in the case of the monomethyltin(IV) moiety, in agreement with literature data for a series of DNA–Main Group metal ion systems. The structural parameters of calf thymus DNA in solution determined by SAXS remain unchanged in the presence of all the methyltin(IV) species studied here. It can be concluded that only when cationic organotin species are present in solution can an interaction through the phosphate groups of DNA be established. SAXS results for dilute solutions suggest that bonding of $SnMe_n$–DNA, in the appropriate range of pH, mainly involves isolated DNA chains; this is also presumed to be so in the DNA condensates obtained if large amounts of reactants are involved.

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