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A proton nuclear magnetic resonance study of the interaction of zinc(II) with the antitumour drug streptonigrin

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The interaction of zinc(II) with the antitumour drug streptonigrin in [$^2H_8$]tetrahydrofuran has been studied by 400 MHz variable-temperature ^1H NMR spectroscopy. At 190 K, and in the presence of an excess of zinc(II) trifluoromethanesulfonate (triflate) (10–15 equivalents), streptonigrin forms a 1:1 complex **1a** (6%) and two diastereomeric 2:1 complexes, **1b** (15%) and **1c** (79%). The relative concentrations of these complexes are highly temperature dependent and at room temperature only **1a** is detected. A fourth minor complex **1d** was detected (< 5%) in the range 240–260 K. In CD_3CN and less than 5 equivalents of zinc(II) triflate, **1a**–**1d** are detected, but in the presence of 10–15 equivalents of zinc(II) triflate **1a** is the predominant complex (> 93%) in the range 235–300 K. Implications for the role of metal ions in the mechanism of the antitumour action of streptonigrin are discussed.

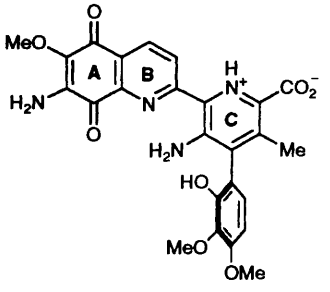
Streptonigrin is a functionalized 7-aminoquinoline-5,8-dione which is highly active against a range of human cancers.[1–6] In common with other aminoquinone antitumour antibiotics, extensive studies suggest that streptonigrin exerts its antitumour activity by causing DNA strand scission.[7–9] Metal ions appear to play a crucial role in this process, either by direct complexation with streptonigrin or reduced streptonigrin[8–12] and/or catalysing the production of hydroxyl radical[7–9] in a series of redox reactions involving several species including streptonigrin semiquinone and superoxide.

Owing to their biological relevance, spectroscopic studies of the interaction of metal ions with streptonigrin have been reported.[10 14] However, there are no structural data on *any* of the metal complexes of streptonigrin. Potentiometric titrations of streptonigrin in buffered solutions (pH 5–8) in the presence of equimolar amounts of Zn^{II}, Cu^{II}, Cd^{II} and Mn^{II} are consistent with the formation of 1:1 complexes, accompanied by the release of 1 mol of protons per mol of bound metal.[13] Additional metal binding occurs beyond the point at which the first proton displacement is completed[12,13] and it has been suggested that the existence of multiple binding sites may play a role in the biological function of streptonigrin.[12,13]

Zinc(II)–streptonigrin complexes appear to have unique properties compared with other transition metal–streptonigrin complexes. Reduction of the quinone ring of streptonigrin is inhibited by Zn^{II} in acetonitrile, and this effect has been suggested to be due to a zinc-assisted tautomeric shift.[13] A stable zinc–streptonigrin-DNA complex has been reported,[12] suggesting that Zn^{II} may act as a delivery agent for streptonigrin to DNA. The presence of Zn^{II} also increases the binding of chemically reduced streptonigrin to DNA.[10] Owing to the particular importance of Zn^{II} in the biological activity of streptonigrin, the present study focused on the determination of the relative amounts and stabilities of the zinc chelates of streptonigrin in organic and aqueous solutions. The zinc chelates were structurally characterized using ^1H NMR spectroscopy. Establishing the structures and stabilities of these complexes is important in understanding the metal-induced cleavage of DNA by streptonigrin.

Results

In polar solvents streptonigrin exists predominantly as the zwitterion with rings A–C coplanar, held in place by a hydrogen bond between the amino group on ring C and the pyridyl nitrogen in ring B.[15] Streptonigrin is optically active in solution,[16,17] which has been attributed to restricted rotation about the aryl bond connecting rings C and D as a result of severe steric hindrance between the amine and methyl groups of ring C and the hydroxyl group of ring D. The absolute configuration has been determined to be (*R*),[17] on the basis of circular dichroism (CD) spectra of derivatives.† The barrier to rotation about the aryl bond connecting rings C and D is very high ($\Delta G^{\ddagger}$ = 102.9 ± 0.3 kJ mol^{-1} at 37.7 °C estimated using CD spectroscopy). Equilibration can be followed by monitoring the change in circular dichroic absorption (ellipticity) with time at a given temperature.



Streptonigrin

NMR titration experiments

A series of variable-temperature ^1H NMR experiments were carried out with zinc(II) trifluoromethanesulfonate (triflate) which, on complexation with streptonigrin, gives a very soluble dark red brown complex(es). Fig. 1 shows the ^1H NMR spectra obtained on addition of zinc(II) triflate to streptonigrin (10.0 mmol dm^{-3}) at low temperature in [$^2H_8$]tetrahydrofuran. In addition to the resonances due to streptonigrin, new resonances appeared which increased in intensity until, when 10–15 equivalents of Zn^{II} were present, no resonances due to uncomplexed streptonigrin remained [Fig. 1(*c*)]. Integration of the assigned resonances H^3, H^4, OH [Fig. 1(*c*)] indicated the presence of three complexes **1a**–**1c**. The relative concentrations

† The optical activity of streptonigrin was reported in ref. 15. It was suggested that the absolute configuration was (*S*), based on a shorter-wavelength Cotton effect observed in the CD spectrum of streptonigrin in ethanol.

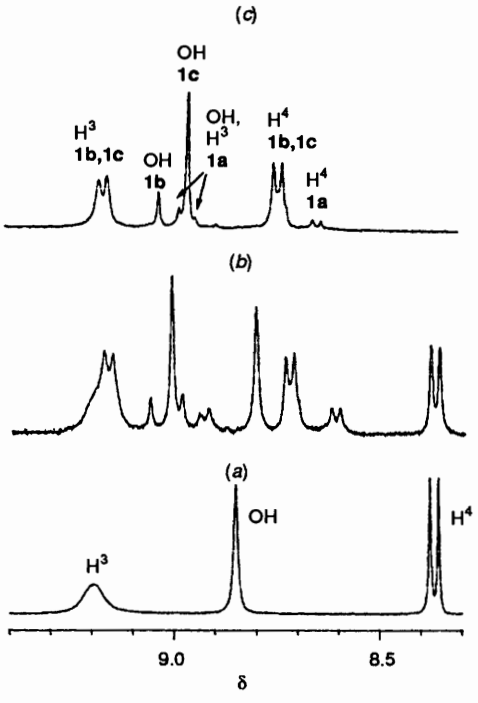


Fig. 1 Portions of the 400 MHz ^{1}H NMR spectra ([$^2H_8$]tetrahydrofuran, 190 K) of (*a*) streptonigrin, (*b*) streptonigrin + 3 equivalents $Zn(SO_2CF_3)_2$ and (*c*) streptonigrin + 12 equivalents $Zn(SO_3CF_3)_2$

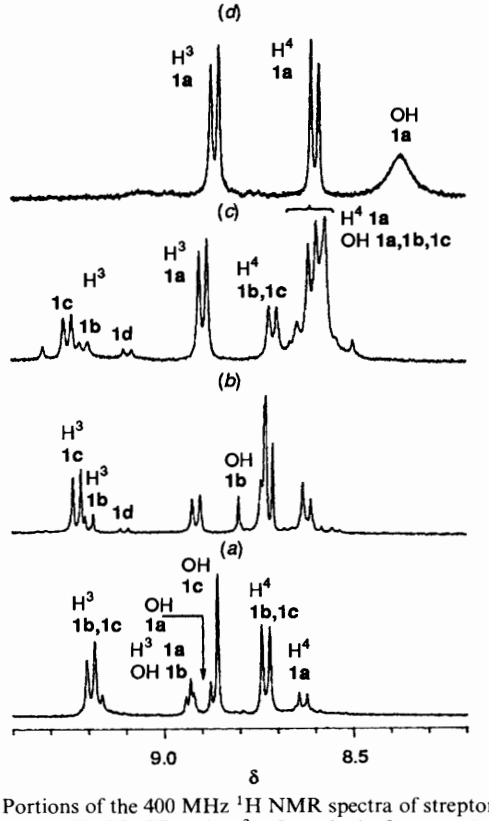
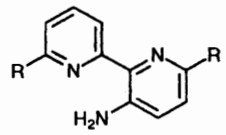


Fig. 2 Portions of the 400 MHz ^{1}H NMR spectra of streptonigrin + 12 equivalents $Zn(SO_3CF_3)_2$ in [$^2H_8$]tetrahydrofuran at (*a*) 214 K, **1a**:**1b**:**1c** = 15:9:76, (*b*) 243 K, **1a**:**1b**:**1c**:**1d** = 36:13:48:3, (*c*) 271 K, **1a**:**1b**:**1c**:**1d** = 63:5:28:3 and (*d*) 305 K, **1a** only; ratios obtained by integration (± 5%)

of these complexes were highly temperature dependent (Fig. 2) and at 305 K only **1a** was detected. In the temperature range 240–260 K peaks due to a fourth species **1d** were observed; the concentration of this complex did not exceed 5% throughout the variable-temperature experiment.



L^1 R = Me
L^2 R = CO_2Me

Assignment of complexes

We have previously reported the characterization of the zinc(II) complexes of 3-amino-6,6′-dimethyl-2,2′-bipyridine (L^1) and 3-amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine (L^2), which contain the key metal-binding subunits of streptonigrin.[18–20] In the case of L^1 and an excess of Zn^{II} the 2:1 (ligand:metal) complex is more stable than the 1:1 complex. In contrast, substantial stabilization of the 1:1 complex of L^2 occurs due to co-ordination of the ester groups at the 6,6′ positions.

Owing to the (*R*) configuration of streptonigrin two 2:1 diastereomeric metal complexes may be formed in which the tetrahedral zinc(II) centre has either the (*R*) or the (*S*) configuration. On comparison of the chemical shifts[19] of the zinc complexes of L^1 and L^2 with those observed for the zinc complex of streptonigrin (Fig. 2), complex **1a** was assigned as the 1:1 bipyridyl complex, while **1b** and **1c** were assigned to the two 2:1 bipyridyl complexes. In particular, H^3 in **1b** and **1c** resonated further downfield than H^3 in the 1:1 complex **1a**. The relative stabilities of the two 2:1 complexes **1b** and **1c** were different with **1c** always present at a significantly higher concentration than **1b**. The difference in stabilities of these complexes is most probably due to steric effects which result when two bulky streptonigrin ligands are co-ordinated to the same metal centre. Corey–Pauling–Koltun (CPK) models of the diastereomers were compared, but **1b** and **1c** could not be assigned to specific stereoisomers.

The presence of a minor amount of a fourth complex **1d** was observed in the range 245–265 K. This may arise due to the presence of five- or six-co-ordinate zinc complexes as the temperature is raised. Alternatively, the presence of minor amounts of (*S*)-streptonigrin in solution, which would give rise to other diastereomeric complexes, was considered. However, the same relative concentrations of **1a**:**1b**:**1c**:**1d** were observed in independent experiments carried out with different batches of (*R*)-streptonigrin consistent with streptonigrin of constant optical purity. A reference experiment was carried out with (*R*,*S*)-streptonigrin and Zn^{II}. In this case four sets of enantiomers are possible, and as expected new signals, as well as those due to **1a**–**1d**, were observed consistent with the formation of the 1:1 complex and four 2:1 diastereomeric complexes.*

An attempt was made to confirm the stoichiometry of the complexes using electrospray mass spectrometry. A strong peak due to the ligand was observed and no molecular ions corresponding to any complexes were detected, confirming the unstable nature of the complexes.

* Attempts to develop an NMR assay to measure the optical purity were unsuccessful. Circular dichroism provided an estimate of the purity but was not accurate enough to quantitate optical purity. An optically inactive sample of streptonigrin was prepared by heating an ethanol solution at 60 °C for 18 h. A range of chiral shift reagents, tris{(3-heptafluoropropylhydroxymethylene)-D-1,7,7-trimethylbicyclo[2.2.1]heptan-2-onate}-, tris{3-(*tert*-butylhydroxymethylene)-D-1,7,7-trimethylbicyclo[2.2.1]heptan-2-onate}- and tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato)-europium(III), and (*R*)-(+)-*tert*-butyl(phenyl)phosphinothioic acid, were added to this sample, but failed to resolve the enantiomers of streptonigrin. The preparation of diastereomeric salts by reaction of streptonigrin with the chiral acid chlorides (*S*)-(−)-*N*-(trifluoroacetyl)-prolyl chloride and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorde in the presence of base did not give conclusive results.

Effect of solvent

While most studies were carried out in [$^2H_8$]tetrahydrofuran, in order to compare the results with the solution conformation of streptonigrin [15] the effect of CD_3CN was also examined. The same trends were observed as in the case of the metal complexes of both L^1 and L^2,[19] and the relative stabilities of the complexes were strongly dependent on the nature of the solvent.

Titration of zinc(II) triflate with a CD_3CN solution of streptonigrin at 235 K gave rise to complexes **1a**–**1d**. Addition of 0.5 equivalent of Zn^{II} to streptonigrin (> 3 mmol dm^{-3}) at 235 K gave predominantly the 2:1 complexes, **1b** and **1c** (83%). Addition of 1.0 equivalent of Zn^{II} decreased the relative concentration of the 2:1 complexes to 72%. This was accompanied by an increase in the concentration of the 1:1 complex, **1a**. This trend continued with **1a** present at 93% on addition of 10.0 equivalents of Zn^{II} at 235 K, and only **1a** was detected at 300 K. Under all conditions studied the relative concentration of **1a** increased with increasing temperature. With dilute solutions of streptonigrin (< 3 mmol dm^{-3}) the 1:1 complex, **1a**, was the only species observed under all experimental conditions investigated.

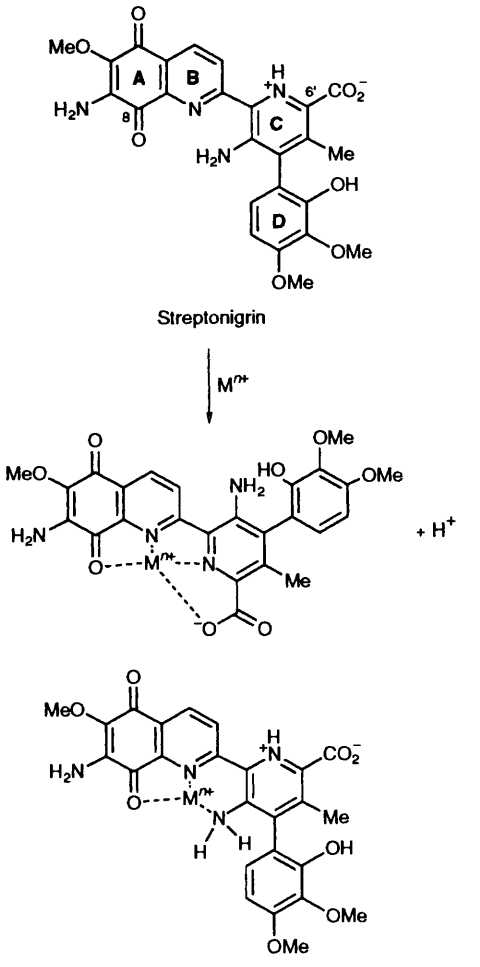
These results are consistent with the solvent effects observed for L^1 and L^2.[19] Solvents of higher donor number (*e.g.* tetrahydrofuran, MeOH, water) compete more strongly with streptonigrin for interaction with Zn^{II}, hence lowering the stability of the zinc(II) complexes of streptonigrin. In these solvents an excess of Zn^{II} is required to achieve complete complexation, and in the range 260–305 K the major species present is the 1:1 complex **1a**.

Discussion

Streptonigrin contains many potential chelation sites including the pyridine-2-carboxylic, the 2,2′-bipyridyl, the aminoquinone, the 2-(3-amino-2-pyridyl)quinoline systems and the amino and phenolic groups on rings C and D. On the basis of spectroscopic data, and changes in the chemical properties of streptonigrin as a result of metal–ligand interactions, streptonigrin has been suggested to form two types of complexes in which the metal ion co-ordinates either to the bipyridyl nitrogens, or to the pyridyl nitrogen in ring B and the amino nitrogen of ring C (Scheme 1).[13] Our own studies on the metal chelates of L^1 and L^2 suggested that both copper(II) and zinc(II) favour bipyridyl co-ordination in streptonigrin.[18–20] Studies on the model ligands also suggested that the oxygen substituents at positions 8 and 6′ of streptonigrin will have a significant role in stabilizing metal complexes of the drug.

The results of the present study in tetrahydrofuran show that with Zn^{II}, and at low temperatures, streptonigrin forms 1:1 and 2:1 complexes. At 190 K, and in the presence of an excess of zinc(II) triflate, streptonigrin forms the 1:1 complex **1a** (6%) and two diastereomeric 2:1 complexes **1b** (15%) and **1c** (79%). At room temperature, the 2:1 complexes are destabilized and only the 1:1 bipyridyl complex is present in solution. Of particular relevance is the structure of the complexes under biological conditions. Streptonigrin has been administered to patients [2,3] in both saline and glucose solutions. To our knowledge, metal complexes of the drug have not been administered, and formation of metal chelates *in vivo* occurs due to scavenging of ions after injection. At 37 °C, in aqueous solution, our results show that the 1:1 bipyridyl chelate is the only complex that needs to be considered.

Several examples of the selective recognition of DNA by chiral metal complexes have been reported, notably octahedral ruthenium complexes of phenanthroline and phenazine.[21] The overall shape or handedness of these complexes matches the shape of right-handed DNA, with one enantiomer recognizing and binding to right-handed DNA. In the case of streptonigrin the formation of a chiral metal complex may also be important



Scheme 1

in DNA recognition. While the exact role of the metal ions in the mechanism of metal-ion-induced cleavage of DNA by streptonigrin remains speculative, one hypothesis is that complexation affords a positively charged complex which may interact favourably with the negatively charged phosphate backbone.[9] In this case the metal ion acts as a delivery agent which positions streptonigrin (or reduced streptonigrin) close to the sugar backbone for radical cleavage upon activation of the quinone ring (ring A).[9] 'Footprinting' of streptonigrin and its metal complexes has not been reported, but preliminary data on copper(II)-induced DNA strand scission suggests that there are preferred cleavage sites.[11] The chirality of streptonigrin and streptonigrin metal complexes may be important in placement of the drug at specific sites on DNA.

Experimental

The NMR spectra were recorded on a Bruker AMX400 spectrometer over spectral widths of 8000 Hz with quadrature detection and referenced to the residual solvent proton resonances at δ 1.7. The spectrometer temperature was calibrated according to the shift difference in methanol.[22] Streptonigrin was obtained from the Sigma Chemical Company and used as provided. Zinc(II) triflate was dried under high vacuum at 100 °C for 2 d prior to use. Water was purified using a Millipore Alpha-Q system. Ethanol was distilled prior to use.

Titration experiments

The NMR samples of streptonigrin were prepared as 2, 6.5–10.0 or 20 mmol dm^{-3} solutions. Titration of zinc(II) was carried out by addition of a solution of zinc(II) triflate in the relevant

deuteriated solvent ([$^2H_8$]tetrahydrofuran or CD_3CN) or by addition of solid to the NMR tube and vortexing of the solution. Both methods gave the same results.

Circular dichroism experiments

Circular dichroism measurements were made on a JASCO-720 spectrometer, using a Neslab RTE-111 water-bath for temperature control and a Digi-Sense thermocouple to record the temperature inside the cell. The instrument was calibrated using a standard solution of aqueous ammonium (*S*)-(+)-7,7-dimethyl-2-oxo-bicyclo[2.2.1]heptane-1-methanesulfonate (0.6 mg cm^{-3}) in a 1 cm cell (CD = 190.4 mdeg at 290.5 nm). All spectra were recorded using a 1 cm cell and the following parameters: λ = 200–500 nm, step 0.5 nm, speed 0.5 nm min^{-1}, response 1 s, bandwidth 1 nm. The relative optical purity of each streptonigrin sample was determined by CD measurement (differential absorption coefficient, $\Delta\varepsilon$) of a 10^{-5} mol dm^{-3} solution in ethanol at ambient temperature. An ethanol blank was used as a reference.

The barrier to rotation of the C–C bond between rings C and D in streptonigrin was estimated in water at 37.7 and 50.0 °C. The CD ellipticity (mdeg) and $\Delta\varepsilon$ (cm^2 $mmol^{-1}$) were recorded at 252.5 and 309.0 nm at 5 min intervals over 5 h. A water blank recorded prior to and on completion of each variable-time experiment showed no significant changes, and was used as the reference. The rate constant (k) was calculated for the equilibrium $R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S$, where $k_1 = k_2 = k$ assuming a single transition state. Solving the relevant differential equation gives the expression $\theta_t = \theta_0 \exp(-2kt)$ where θ_t = observed CD (mdeg) at time t in s and θ_0 = initial CD (mdeg). The CD data were fitted by this equation to give the rate constant k.

Acknowledgements

Financial support from the Sydney University Cancer Research Fund (to M. M. H.), an Australian Postgraduate Research Award and a Sydney University Henry and Mabel Gritton Supplementary Postgraduate Scholarship (to G. V. L.) are acknowledged.

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Received 7*th August* 1995; *Paper* 5/05278E