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Preparation and ESR spectroscopic characterization of the zinc(II) and cadmium(II) complexes of streptonigrin semiquinone¹

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Abstract

Semiquinone metal complexes derived from the antitumor antibiotic streptonigrin have been prepared for the first time. They were obtained by reduction of the zinc(II) and cadmium(II) complexes of the parent aminoquinone ligand with sodium borohydride, followed by air oxidation of the intermediate dihydroquinones. Alternatively, *N*-benzylidihydronicotinamide reduction was used to produce the same Cd(II) complex of the *p*-semiquinone free radical. Electron spin resonance spectroscopic studies showed that metal binding significantly changes the spin densities of the unpaired electron which is confined to the quinolinesemiquinone moiety of the complexed antibiotic. Complexation with both Cd(II) and Zn(II) perturbs the coupling constants of all atoms involved in delocalization of the unpaired electron, shifting its distribution toward the pyridine ring. The coupling constant of the pyridine ring-proton adjacent to the semiquinone ring increases from 0.31 to 0.43 G in the Cd(II) complex in methanol, while the proton meta to the pyridine nitrogen increases from 1.76 to 1.96 G. Furthermore, the coupling constant of the heterocyclic nitrogen increases from 0.46 to 0.61 G. A similar trend is noted for the Zn(II) complex as well, including the observed decrease in splitting constant of the amino nitrogen from 1.34 to 1.08 G, and perturbation of the previously equivalent amino protons from 0.89 and 0.89 to 1.09 and 0.95 G. The spectral parameters have been confirmed by deuteration. Complexation studies using isotopically enriched ¹¹³Cd(II) revealed hyperfine coupling of the unpaired electron of the *p*-semiquinone and the nuclear spin of ¹¹³Cd(II), indicating direct coordination between the metal and the complexing ligand. Although the metal complexes could readily be prepared in a series of different solvent systems, they appear to have substantially shorter half lives than the non complexed *p*-semiquinone radical (5 to 15 min vs. 2 to 3 wk in sealed ampoules). Formation of tight-binding *p*-semiquinone metal complexes as here described should provide useful leads for the design of related systems to study *p*-quinone-metal interactions for mechanistic elucidation of metal ion catalyzed quinone-dependent electron transfer reactions in biological oxidations. © 1997 Elsevier Science B.V. All rights reserved.

Keywords: Streptonigrin; Quinolinesemiquinone; *p*-Semiquinone metal complex; Spin-spin coupling; Aminoquinone antibiotic

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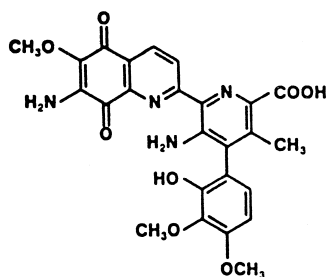
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1. Introduction

Quinoenzymes are a widely distributed class of redox enzymes in which the quinone cofactor frequently occurs in conjunction with metal ions as part of the catalytically functioning complex [1,2]. Structural characterization of metal-quinone complexes and mechanistic elucidation of the role of the metal ion in influencing the reactivity of the redox active coordinating ligand, therefore, represent a fundamental chemical problem of considerable biological significance [1,3–6]. Methoxatin, for example, was first recognized to be a cofactor in methyltropic bacteria [7], where it has been suggested to serve in place of nicotinamide-cofactor requiring enzymes and flavoenzymes in the oxidation of alcohols, hexoses, aldehydes and methylamine [2]. Subsequently, it was found as a cofactor in *E. coli* [8,9], and has also been suggested to function as a cofactor for mammalian plasma amine oxidase [10].

While the chemistry of transition metal *o*-quinone and semiquinone complexes has attracted considerable attention [11], much less information is available on *p*-quinone and semiquinone complexes [3–6,12,13]. The biochemical importance of the latter is well documented: plastoquinone, a substituted *p*-benzoquinone is known to be involved in electron transfer from photosystem II to photosystem I in green plants [14] while ubiquinone, another highly substituted *p*-benzoquinone interacts with the high-potential iron-sulfur protein in mitochondria [15]. Furthermore, an iron-ubiquinone complex has also been implicated as electron acceptor in bacterial photosynthesis [16].

In addition to the importance of quinones and their functioning in conjunction with metal ions in normal biological redox reactions, *p*-quinones [17] and metal-*p*-quinone complexes have been implicated in the mechanism of action of pharmacologic agents [18,19]. Along these lines streptonigrin (1),



(1)

an antitumor active antibiotic produced by *Streptomyces flocculus* has been shown to require metal ions for activity [18,19]. There is strong evidence that activation of the aminoquinone moiety through its semiquinone-metal complex is a prerequisite for the *in vivo* antitumor action of the antibiotic [20]. Specifically, while formation of radicals in bacterial systems [21], microsomal preparation [22] as well as enzymological studies [23] has been demonstrated, identification and characterization of the free radical intermediate and its role in the mechanism of antitumor-action of the antibiotic remain to be elucidated [24]. Evidence indicates that streptonigrin has as its principal target site the nucleic acids and causes single-strand breakage of DNA [20]. Furthermore, it has been reported that streptonigrin interferes with the cell respiratory mechanism as well [20]. Both mechanisms are thought to involve participation of metal ions [18] and require an electron source, as well as oxygen [24]. Although spectrophotometric studies have indicated complex formation of streptonigrin with Zn(II), Cu(II), Cd(II) or Mn(II) [24,25], the structure of the complexes as well as the catalytic role of metal ions in promoting reactions with DNA and proteins remain to be determined. Participation of metal ion may involve (1) stabilization of streptonigrin-semiquinone, (2) direct interaction with O₂ leading to a M-O₂ adduct, or (3) facilitating electron transfer in reduction of oxygen by the reduced antibiotic [26]. Furthermore, metal ion could also promote association between DNA and the drug [27].

Beyond its intrinsic importance as a member of the highly potent aminoquinone antibiotic family, streptonigrin can serve as a suitable model system to study metal-quinone interactions since it contains a tight-binding chelating moiety capable of bringing the complexed metal ion in close proximity to the redox active quinolinequinone function of the molecule.

As part of our investigation of streptonigrin metal-complexes we have set out to prepare streptonigrin semiquinone and its complexes with diamagnetic metal ions to determine the ESR spectra of the radicals under a series of well-defined reaction conditions in order to gain insight into the physical and chemical properties of these species. Specifically, ESR spectroscopy should become a powerful tool for the study of complexation of streptonigrin semiquinone with various metal ions in solution. Complexation studies with diamagnetic metal ions (such as Cd(II) and Zn(II)) is expected to give two

types of structural information: (1) modification of spin density distribution within the ligand and (2) hyperfine coupling to the nuclear spin of the metal. For the latter, isotopic enrichment is necessary since main isotopes of Cd(II) and Zn(II) are non-magnetic. Furthermore, these studies should open the way for subsequent experiments to obtain structural information from complexation with paramagnetic metal ions (such as Cu(II)) since interaction between the unpaired electron of the ligand and that of the metal will affect the ESR spectrum due to spin-spin coupling [28,29].

2. Materials and methods

2.1. Solvents

Dimethyl sulfoxide (DMSO) and methanol (spectrograde) were purchased from Burdick and Jackson and used without further purification. Methanol- d_4 was obtained from Sigma and used as received. 1,4-Dioxane (99%) was purchased from Fisher and purified by passing it through neutral alumina (J.T. Baker #0537) before use. Deuterium oxide (99.8%) was purchased from ICN Biomedicals.

2.2. Reagents

Streptonigrin (NSC #45383) was a generous gift of Dr. John D. Douros, Drug Research and Development, Chemotherapy, National Cancer Institute. Lithium hydroxide monohydrate was purchased from Matheson Coleman and Bell (MC and B). Lithium deuteride was prepared by deuterium exchange from 0.1 M LiOH · H₂O in D₂O followed by freeze-drying (deuteration was confirmed by ¹H-NMR). Sodium borohydride (98%, Stern Chemicals) and sodium borodeuteride (99%D, Chemical Dynamics Corporation) were used as received. *N*-Benzylidihydronicotinamide was prepared as described [30]. ¹H-NMR, TLC and UV absorption analyses confirmed its structure as reported [31]. Cadmium acetate dihydrate (reagent grade) was obtained from MC and B, zinc chloride (anhydrous, ultrapure) was purchased from Alfa Products. Cadmium-113 oxide (93.4 atom%) was obtained from MSD Isotopes, Montreal (Canada). Cadmium-113 acetate was prepared by dis-

solving 30.0 mg cadmium-113 oxide in a mixture of 7.5 ml glacial acetic acid (Mallinckrodt) and 7.5 ml deionized water, followed by freeze drying. The crystalline product was redissolved in 20 ml deionized water, then freeze-dried two more times.

2.3. Methods

2.3.1. Determination of the ESR spectra

ESR spectra of the radicals were obtained with a Bruker Series 200 X-band spectrometer employing 100 KHz field modulation interfaced with an IBM 9000 computer. The field sweep was calibrated with an NMR Gaussmeter and the *g*-value measured using a dual cavity technique [32] with peroxyamine disulfonate in water, as a standard *g* = 2.0055 [33]. The source of the microwave radiation was a klystron tube coupled to an attenuator to provide a means of adjusting the microwave power. Care was taken with the use of the microwave power to avoid power saturation which can be determined by power saturation curves. In a power saturation curve, the ESR signal intensity increases linearly as the square root of the microwave power in the absence of saturation; however, when power saturation is achieved, departure from linearity is observed. Spectrometer settings (modulation amplitude, time constant) were adjusted to minimize spectral distortion [34].

The sample, sealed in a 50 μ l disposable pipet, was inserted in the center of a quartz Dewar, which was placed in the center of the resonance cavity of the ESR spectrometer. The temperature of the sample was controlled to the desired value by passing thermostatted kerosene through the quartz Dewar. While the temperature of the kerosene was controlled ($\pm 0.1^\circ\text{C}$) in thermostatted bath (NESLAB Instruments, RTE-5DD), a thermocouple (Bailey Instruments, BAT-9) was inserted into the dewar alongside the capillary sample. This way the sample temperature, which deviated slightly from the external bath temperature, could be measured accurately.

2.3.2. Preparation of semiquinone complexes with Cd(II), Zn(II), and ¹¹³Cd(II)

Semiquinone metal complexes were prepared by reduction of streptonigrin solution containing equimolar metal ions either with NaBH₄ or *N*-benzylidihydronicotinamide as the reducing agent. The

ESR spectra were recorded at 25°C. As metal ions precipitate in strongly basic solutions, the semiquinone metal complexes were prepared under less basic conditions compared to those used for preparation of metal-free streptonigrin semiquinone [35]. Thus, the streptonigrin semiquinone complex of Cd(II) in methanol was prepared by dissolving 2.0 mg ($4.0 \cdot 10^{-6}$ mol) streptonigrin in 2.0 ml methanol containing three drops of 0.1 M LiOH in methanol and 0.04 ml of 0.1 M cadmium acetate stock solution ($4.0 \cdot 10^{-6}$ mol), followed by addition of excess (< 0.2 mg) solid NaBH_4 . The streptonigrin semiquinone complexes with Zn(II) and $^{113}\text{Cd(II)}$ were prepared similarly, using the corresponding stock solution of zinc chloride and isotopically enriched cadmium acetate. Streptonigrin semiquinone complex with Cd(II) in aq. DMSO and aq. 1,4-dioxane was prepared in 0.06 M potassium phosphate buffer pH 11.0 containing 10% DMSO or 50% 1,4-dioxane in a similar fashion, using solid NaBH_4 as reducing agent. The cadmium complex of the semiquinone radical in 10% aq. DMSO was also prepared by reduction with excess solid *N*-benzylidihydronicotinamide (< 2 mg).

All of the streptonigrin metal complex solutions described above showed no precipitation. However, in the absence of streptonigrin, under similar conditions, the metal ions precipitated as evidenced by the turbidity of the solution. The same complexes were also prepared in the presence of excess amounts of metal ion (up to five-fold excess). Deuterated streptonigrin semiquinone metal complexes were prepared similarly, except that deuterated solvents (the pH-meter readings of the buffers being adjusted for pD according to Glasoe and Long [36]) and deuterated reducing agents were used.

2.3.3. Computer simulation of the ESR spectra

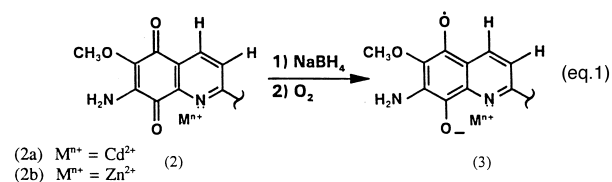
The ESR spectra were simulated using the algorithm of Oehler [37]. A stick diagram is generated according to the input hyperfine coupling constants and a line shape function applied to each stick at the end. The resulting simulated spectra were compared with experimental spectra and were optimized by trial and error. The best fitting line shape consisted of a linear combination of Lorentzian (30% doubly-integrated) and Gaussian each of the same linewidth [38]. The Gaussian character probably results from a combination of unresolved hyperfine structure and

from modulation broadening [39]. In each spectral simulation a constant linewidth was employed for all of the lines; therefore, in more viscous solvents in which hindered rotation led to significant linewidth variation, the fits between the experimental spectra and the simulated spectra are poorer toward the high-field side of the spectra [35].

3. Results

3.1. Formation of streptonigrin semiquinone complexes with Cd(II) and Zn(II)

Reduction of an anhydrous methanolic solution of 2 mM streptonigrin-zinc(II) or streptonigrin-cadmium(II) complex of equimolar metal-to-ligand ratio in the presence of stoichiometric amounts of LiOH with excess solid NaBH_4 followed by air oxidation produces a deep-green solution of the semiquinone-metal complex (3) (Eq. 1).



The ESR spectra of the resulting metal complexes differ from those of the free semiquinone radical in a number of characteristic ways in (Fig. 1). Significantly, in the presence of the metal the 18-line spectrum of the streptonigrin semiquinone (Fig. 1a) is perturbed to a multiline spectrum (Fig. 1b,c). The spectral assignments for the semiquinone-Cd(II) and Zn(II) complexes as well as the coupling constants of the parent semiquinone are shown in Table 1. We have obtained evidence for the formation of streptonigrin semiquinone-Cd(II) complex in three different solvent systems (in methanol, containing $2 \cdot 10^{-3}$ M LiOH, in 10% aqueous DMSO buffered with 0.06 M potassium phosphate pH 11.0 and in 50% 1,4-dioxane in 0.06 M potassium phosphate pH 11.0). Formation of the semiquinone complex with Zn(II) has been studied in methanol containing 2×10^{-3} M LiOH. The semiquinone metal complexes were prepared by adding an aliquot of metal ion stock solu-

tion to 2.0 mM streptonigrin solution yielding equimolar streptonigrin-metal complex, followed by reduction with excess solid NaBH_4 or *N*-benzylidihydronicotinamide. Upon addition of metal ion stock solution the color of the streptonigrin solution turns darker, but remains clear. This is consistent with formation of the streptonigrin metal complex [24,25], as in the absence of streptonigrin the metal ions precipitate under the basic reaction conditions (indicated by the development of turbidity in the solution). The semiquinone Cd(II) complex in aq. 0.06 M potassium phosphate, pH 11.0, containing 10% DMSO has also been prepared using *N*-benzylidihydronicotinamide as reducing agent yielding an identical ESR spectrum to that obtained with sodium borohydride, indicating that the same radical is formed in both cases irrespective of the reducing agent used.

The ESR spectral assignments for the semiquinone-Cd(II) complex in the series of solvent systems employed are presented in Table 2. Although the spectra of the complexes appear to be quite different in the solvent series, determination of the spectral parameters shows relatively modest variation in the magnitude of the individual coupling constants calculated. Comparison of the ESR spectral parameters of the free semiquinone and the semiquinone-metal

complexes shows that complexation with Cd(II) perturbs the coupling constants of all atoms involved in the delocalization of the free electron. The coupling constant of the pyridine ring-proton adjacent to the semiquinone ring (a) increases from 0.31 to 0.43 G in methanol, from 0.27 G to 0.41 G in aq. DMSO and from 0.26 G to 0.45 G in aq. 1,4-dioxane. The coupling constant of the proton meta to the pyridine-nitrogen (a) increases in magnitude from 1.76 G to 1.96 G in methanol, from 1.73 G to 2.04 G in aq. DMSO and from 1.75 G to 2.00 G in aq. 1,4-dioxane. Similarly, the coupling constant of the heterocyclic nitrogen increases on complexation (from 0.46 G to 0.61 G in MeOH, 0.47 G to 0.66 G in aq. DMSO, and 0.43 G to 0.66 G in aq. 1,4-dioxane).

Complex formation also impacts on the equivalence of the amino protons attached to the semiquinone ring. On complexation with Cd(II) in methanolic solution, the two previously equivalent amino protons become non-equivalent changing from 0.89 G and 0.89 G to 1.00 G and 0.93 G, while complexation with Cd(II) in aq. DMSO and aq. 1,4-dioxane enhances the non-equivalence of the two amino protons from 0.89 G and 0.83 G to 1.06 G and 0.94 G in aq. DMSO, 0.80 G and 0.77 G to 0.94 G and 0.86 G in aq. 1,4-dioxane. These results clearly

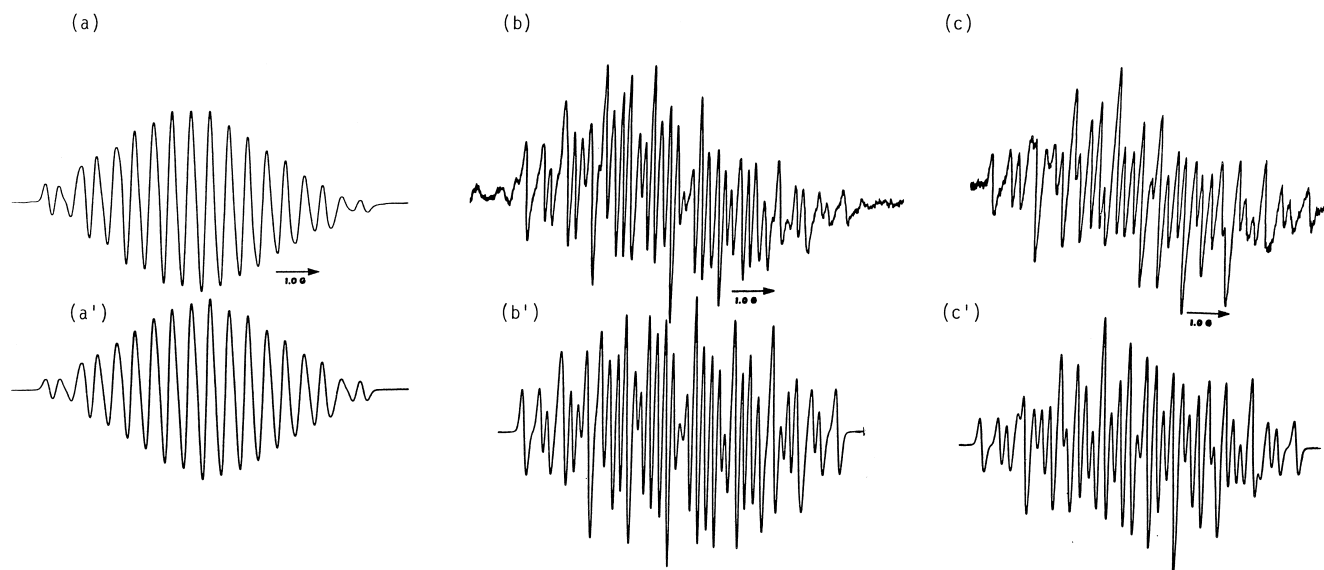
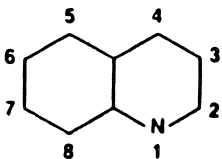


Fig. 1. The effect of metal binding by streptonigrin semiquinone on the ESR spectra: (a) spectrum of the free semiquinone, (b) spectrum of the Cd(II) complex, and (c) spectrum of the Zn(II) complex, all in methanol at 25°C. The corresponding computer simulations (a'), (b'), (c') were obtained using the parameters presented in Table 1.

Table 1

ESR parameters^a for the streptonigrin semiquinone and its metal complexes in methanol and for deuterated streptonigrin semiquinone and its metal complexes in deuteromethanol

Position	Fig.	Solvent	Metal	$a_{\text{NH}_2}^{\text{N}}$	$a_{\text{NH}_2}^{\text{H}}$	$a_{\text{ND}_2}^{\text{D}}$	$a_{\text{NH}_2}^{\text{H}}$	$a_{\text{ND}_2}^{\text{D}}$	a_3^{H}	a_4^{H}	a_1^{N}
	Fig. 1a	MeOH/LiOH		1.34	0.89		0.89		1.76	0.31	0.46
		MeOD/LiOD		1.34		0.14		0.14	1.76	0.31	0.46
	Fig. 1b	MeOH/LiOH	Cd(II)	1.19	1.00		0.93		1.96	0.43	0.61
		MeOH/LiOH	Cd(II)	1.19		0.15		0.14	1.96	0.43	0.61
	Fig. 1c	MeOD/LiOD	Zn(II)	1.08	1.09		0.95		2.00	0.46	0.67
		MeOD/LiOD	Zn(II)	1.08		0.17		0.15	2.00	0.46	0.67

$t = 25^\circ\text{C}$.

$g_{\text{MeOH}} = 2.0043 \pm 0.0001$.

^a Values in Gauss. Estimated error ± 0.02 G except for $a_{\text{ND}_2}^{\text{D}}$ which has an error ± 0.005 G.

All computer simulated spectra use a lineshape that is a linear combination of Lorentzian (30%) and Gaussian (70%). The simulation is a linear isotopic synthesis with constant linewidth which, of course, cannot duplicate the linewidth variation in the experimental spectrum.

indicate that complexation shifts the electron distribution to the pyridine ring. Similar trend in the perturbation of the coupling constants on complexation with Zn(II) in methanolic solution has been observed (Table 1).

Addition of excess metal ion, up to five-fold excess, did not change the ESR spectra previously obtained for the semiquinone-metal (1:1) complex. This confirms that the spectra correspond only to the semiquinone metal complex, rather than a mixture of free- and complexed forms of the semiquinone.

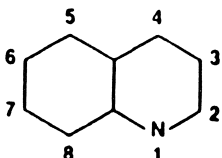
The spectral parameters of the complexes have also been confirmed by deuterium exchange experiments using deuterated streptonigrin [35], solvent [36]

and reducing agents (the coupling constants are summarized in Tables 1 and 2).

Careful examination of the ESR spectrum obtained for the Cd(II) complex of streptonigrin (3a) revealed additional splittings at both ends of the spectrum. These could readily be attributed to hyperfine coupling of the free electron of the semiquinone with the nuclear spin of $^{113}\text{Cd(II)}$ and $^{111}\text{Cd(II)}$ which are present in natural cadmium in 12.34% and 12.86%, respectively [40]. We, therefore, proceeded to prepare the isotopically enriched $^{113}\text{Cd(II)}$ -semiquinone complex. Thus, reduction of 2.0 mM streptonigrin- $^{113}\text{Cd(II)}$ complex in methanolic solution with NaBH_4 indeed produced an ESR signal (Fig. 2a) which has a

Table 2

ESR parameters^a for the cadmium complexes of streptonigrin semiquinone and deuterated streptonigrin semiquinone in a series of solvent systems

Position	Solvent	Metal	$a_{\text{NH}_2}^{\text{N}}$	$a_{\text{NH}_2}^{\text{H}}$	$a_{\text{ND}_2}^{\text{D}}$	$a_{\text{NH}_2}^{\text{H}}$	$a_{\text{ND}_2}^{\text{D}}$	a_3^{H}	a_4^{H}	a_1^{N}	$a_{\text{Cd(II)}}^{113}$
	MeOH/LiOH	Cd(II)	1.19	1.00		0.93		1.96	0.43	0.61	
	MeOD/LiOD	Cd(II)	1.19		0.15		0.14	1.96	0.43	0.66	
	DMSO/H ₂ OD	Cd(II)	1.25	1.06		0.94		2.04	0.41	0.66	
	DMSO/D ₂ O	Cd(II)	1.25		0.16		0.14	2.04	0.41	0.66	
	Dioxane/H ₂ O	Cd(II)	1.17	0.94		0.95		2.00	0.45	0.66	
	Dioxane/D ₂ O	Cd(II)	1.17		0.15		0.13	2.00	0.45	0.61	
	MeOH/LiOH	$^{113}\text{Cd(II)}$	1.19	1.00		0.93		1.96	0.43	0.61	4.32
	MeOD/LiOD	$^{113}\text{Cd(II)}$	1.19		0.15		0.14	1.96	0.43	0.61	4.32

$t = 25^\circ\text{C}$.

^a Values in Gauss. Estimated error ± 0.02 G.

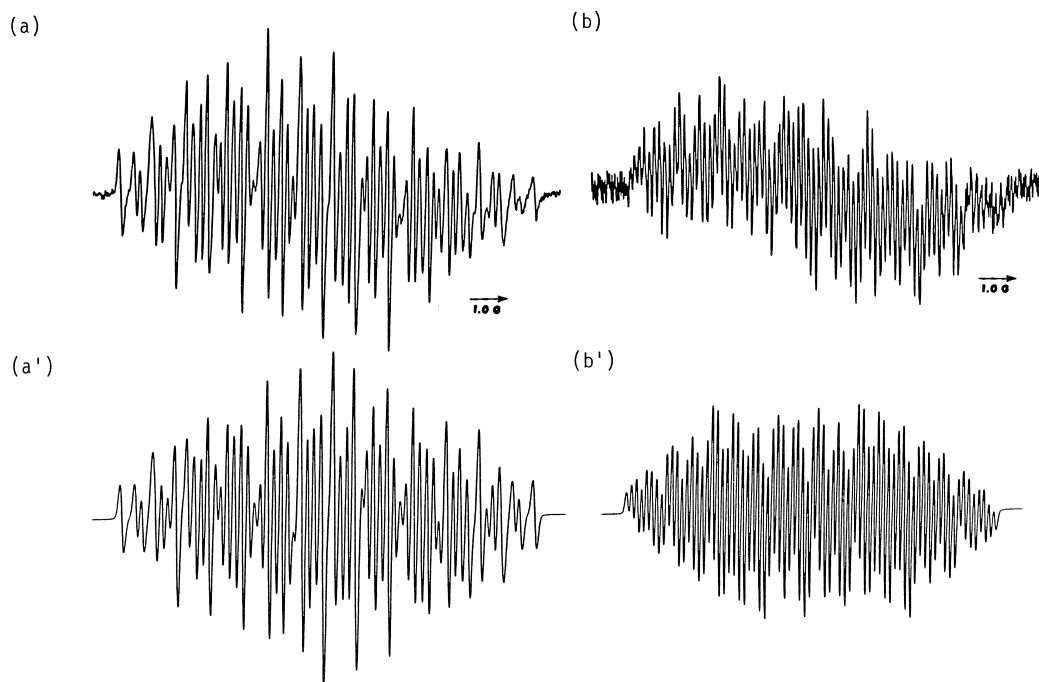


Fig. 2. (a) ESR spectrum of streptonigrin semiquinone complex with $^{113}\text{Cd}(\text{II})$ in MeOH/LiOH at 25°C . Microwave power, 2.0 mW; modulation amplitude, 0.32 G; 18 G sweep in 500 s with a 320 ms time constant. (b) ESR spectrum of deuterated streptonigrin semiquinone complex with $^{113}\text{Cd}(\text{II})$ in MeOD/LiOD at 25°C . Microwave power, 2.0 mW; modulation amplitude, 0.32 G; 18 G sweep in 500 s with a 320 ms time constant. (a') Computer simulation using parameters in Table 2, and a linewidth of 0.13 G. (b') Computer simulation using parameters in Table 2, and a linewidth of 0.11 G.

longer total spread by 4.32 G than the one obtained from semiquinone complex with Cd(II) without enrichment (Fig. 1b). This difference (4.32 G) has therefore been assigned to the coupling constant of the nuclear spin of $^{113}\text{Cd}(\text{II})$ showing a perfect fit with the experimental spectrum obtained from the semiquinone complex with $^{113}\text{Cd}(\text{II})$ (Fig. 2a). This assignment has further been confirmed by deuterium exchange experiments showing good agreement between the experimental and simulated spectra.

Finally, we have observed that the Zn(II) and Cd(II) complexes of streptonigrin semiquinone appear to be less stable than the corresponding metal-free quinoline semiquinone as evidenced by the rapid disappearance of the ESR signal from solutions of the complexes (within 5–15 min, the shortest half-lives having been observed in aq. dioxane, while the longest in aq. DMSO) compared to much longer half-lives (two to three weeks) of the free radical semiquinone in sealed ampoules under similar reaction conditions.

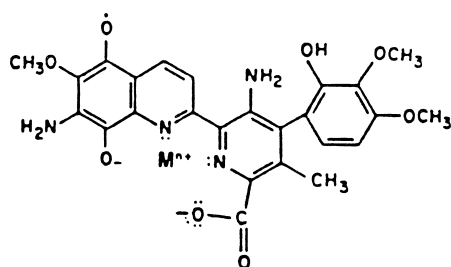
While these observations appear in contrast to the greater stability of the *o*-quinone metal complexes compared to the corresponding *o*-semiquinone free radicals, they underscore the difference in the chemistry and the role of metal ions between the two series [11,13].

4. Discussion

The most important significance of the results here presented is in demonstrating formation of streptonigrin semiquinone metal complexes for the first time. The importance of the metal complexes is twofold: (1) they constitute the antitumor active functional entity of the antibiotic, therefore, elucidation of their chemistry should lead to better understanding of the mechanism of action of this potent antitumor active drug; and (2) they provide a suitable model system to study metal-ligand interactions implicated in quinone

dependent biological redox reactions. Specifically, electron transfer reactions during oxidative energy conversion processes in biological systems have been shown to involve interaction between the metal of a metalloprotein and a *p*-quinone or quinoid electron acceptor [3–6,14,15]. Thus, development of the coordination chemistry of *p*-quinone metal complexes with specific emphasis on preparation and characterization of the corresponding semiquinone intermediates can provide important insight and chemical precedents crucial for understanding the mechanism of electron transfer reactions in quinone-dependent biological oxidations [13].

In contrast to the metal complexes of *o*-semiquinones where the redox-active functional group

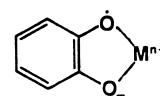


(5a)

arising from the two alternative conformations of the substituted picolinic acid ring.

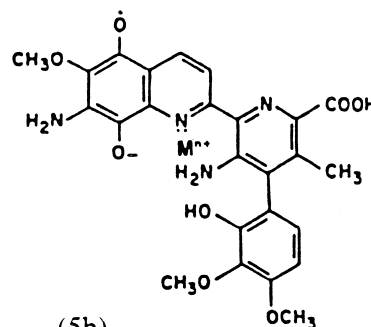
Notwithstanding the remaining ambiguities with regard to the preferred ligand conformation and the nature of the ligand atoms that comprise the rest of the metal-coordination sphere, reduction of the Zn(II) and Cd(II) streptonigrin complexes and ESR spectroscopic characterization of the reduction products have clearly established the formation of *p*-semiquinone metal complexes of 1:1 molar ratio and provided clear indication regarding the effect of complexation on the electron distribution in the redox-active 5,8 quinolinesemiquinone function. Thus, comparison of the ESR spectral parameters of the free semiquinone and the semiquinone metal-complexes suggests that complexation shifts the electron distribution to the pyridine ring, which is consistent with coordination the metal ion by the pyridine nitrogen of the quinolinesemiquinone moiety [20,24,25]. This effect is stronger on complexation with Zn(II) which is consis-

itself provides the chelating function involving the two neighboring oxygen atoms (4)



(4)

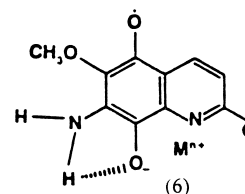
for the *p*-semiquinone series an adjacent metal binding site is necessary to place the metal ion in close proximity to the reaction center. These requirements are quite satisfactorily met by either one of the two potential metal-binding sites of streptonigrin (5a, 5b)



(5b)

tent with the smaller ionic radius of Zn(II) compared to Cd(II).

Furthermore, on complexation with Cd(II) and Zn(II) in methanol, the two previously equivalent amino protons become non-equivalent. This phenomenon might readily be explicable in terms of hydrogen bond formation between one of the amino protons with the anionic semiquinone oxygen (6).



(6)

This hydrogen bond formation should be facilitated by stabilization of the negative charge on the semiquinone-oxygen next to the amino group by the

positive charge of the complexing metal ion. Increased hydrogen bonding should slow down the rotation of the amino group that in turn would result in non-equivalence of the two amino protons as observed (Table 1). Furthermore, such hydrogen bond formation is likely to increase the polarity of the N-H bond of the hydrogen donor amino group, leading to increased partial negative charge on the amino nitrogen, which in turn should decrease electron delocalization resulting in decreased spin density on this nitrogen as observed (Table 1). Similarly, complexation with Cd(II) in aq. DMSO and aq. dioxane exhibits parallel behavior.

Comparison of the ESR spectra of the semiquinone-Cd(II) (without enrichment) complex with that of the semiquinone- $^{113}\text{Cd(II)}$ complex clearly shows the additional splittings due to hyperfine coupling of the free electron of the semiquinone with the nuclear spin of $^{113}\text{Cd(II)}$. The hyperfine coupling provides unambiguous evidence for direct coordination of the metal ion to the 5,8-quinoline-semiquinone radical. Delocalization of the electron to the metal ion, however, is small, as the assigned coupling constant for $^{113}\text{Cd(II)}$ (4.32 G) is much smaller (0.08%) than the coupling constant of $^{113}\text{Cd(I)}$ (5374 G, with one electron occupying the s orbital), obtained on photoirradiation of ^{113}Cd metal atom at 4 K [40]. It is important to point out in this context that comparable magnitude of delocalization of the free electron to the coordinating metal has also been reported for *o*-benzosemiquinone complex with $^{59}\text{Co(III)}$ [41].

In conclusion, preparation and structural characterization of the metal complexes of streptonigrin semiquinone should present useful contribution toward elucidation of the redox chemistry of streptonigrin and at the same time provide a suitable model to study metal-quinone interactions implicated in photosynthesis and biological electron transport.

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