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Anthracycline–Copper(II) Complexes: Structure-dependent Coordination Pattern as Evidenced by ESR

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Abstract

The copper(II) coordination properties of different anthracycline antitumor agents were studied by ESR spectroscopy. Three classes of drugs have been identified: (i) adria-like, which contains adriamycin, daunomycin, 4′-deoxydaunomycin, 4′-deoxy-4′-iododaunomycin and 4-demethoxydaunomycin; (ii) 4′-epiadria-like, which contains 4′-epiadriamycin, 4′-epidaunomycin, 4-demethoxy-6-deoxydaunomycin and 4-demethoxy-11-deoxydaunomycin; and (iii) border-line type, which contains carminomycin, 6-*O*-methylcarminomycin, 11-deoxycarminomycin and 6-deoxycarminomycin. They have been characterized by their different Cu(II) chelation properties.

The adria-like class members give rise to multinuclear complexes, where both the C=O and C–OH functionalities of both sides of the hydroxyanthraquinone system are involved in the coordination. By contrast, the 4′-epiadria-like drugs form monomeric Cu(II) complexes because of the unavailability of the C(6)–OH group. The border-line type compounds yield more than one monomeric Cu(II) adduct because of the presence of OH at the C(4) position.

All Cu(II) derivatives are characterized by a very strong ligand field, the unpaired electron lying in the $d_{x^2-y^2}$ orbital. The onset of complexation is oxygen dependent and this is related to the σ and π bond properties of the anthracycline ligand.

Introduction

Anthracycline antitumor agents have gained wide acceptance in the treatment of various types of human neoplasies [1]. The recognition of some serious dose-dependent side effects [2] has prompted considerable synthetic efforts aimed at minimizing this drawback by modifying the substitution pattern of the anthraquinone and/or sugar moieties [3].

Many mechanistic studies have also been carried out in order to understand and/or better define the mode of action of this class of drugs and ultimately provide useful hints for rational molecular modifications. Beside the intercalative mechanism [4], the functionalities present on the anthraquinone moiety have required that other possible interactions needed to be considered. It has been inferred that the ease of reduction of the quinone–hydroquinone functionality has some bearing on the mechanism of action as, in the presence of enzymes, the drug may participate in and interfere with the cell redox processes [5–8].

The very same functional groups responsible for the redox behavior enable the molecule to become engaged in metal ions complexation equilibria. Since the first observation by Calendi *et al.* [9] two decades ago, relatively few studies have been carried out on the metal ion complexation properties of the anthracyclines and in most of them spectrophotometric techniques (UV–Vis, CD, IR-Raman) were used [10].

Contrasting reports have been published on the dependence of the stoichiometry of the complexes on pH. For instance, according to Dabrowiak and Greenaway [11] a 1:1 drug/Cu(II) complex forms at pH 5.5, whereas in the pH range 7–8.2 the 2:1 species is more stable. Recent work by Garnier-Suillerot *et al.* [12] has defined an 'inverse' stoichiometry under similar experimental conditions. Besides the still undefined stoichiometry, both the geometry of the coordination site and the electronic perturbation induced by the anthracycline ligand field on the metal have not so far been characterized.

Since an understanding of the diverse structure dependent chelation pattern is deemed to be important in view of the likely implications, as yet not identified or studied, of the chelated drugs in the anthracycline redox behavior, we have addressed this topic by carrying out an extensive ESR study

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of the complexation properties of copper(II) with several representative anthracycline analogues.

It has been possible to define the structural requirements leading to the formation of polymeric and/or mononuclear copper(II) derivatives and to study the role of the molecular oxygen in stabilizing the two different types of complex. The latter property is believed to be relatable to the redox metabolism of drugs in the presence of copper(II) ions. The copper–drug interaction mode could mimic the same interaction with copper-containing enzymes [13] and help to explain the expected variations in the functionality of the enzymes in the presence of anthracyclines.

Experimental

The anthracyclines **(I–XIII)** used in this study were provided by Farmitalia C. Erba S.p.A. The purity of each analogue was checked by reverse phase HPLC (HP 1090) on Microbore columns (100 X 2.1 mm) thermostated at 40 °C. (H_2O phosphate buffer, pH = 3.5; CH_3CN). As the anthracycline solutions are sensitive to light, stock solutions were prepared just before use. Standard Cu(II) solutions (*ca.* 10^{-3} M) were prepared from reagent grade $CuCl_2 \cdot 2H_2O$ (Carlo Erba) and mixed with the same volume of an equimolar anthracycline solution to obtain a 10^{-3} M final solution having a drug-to-metal ratio of $r = 1$.

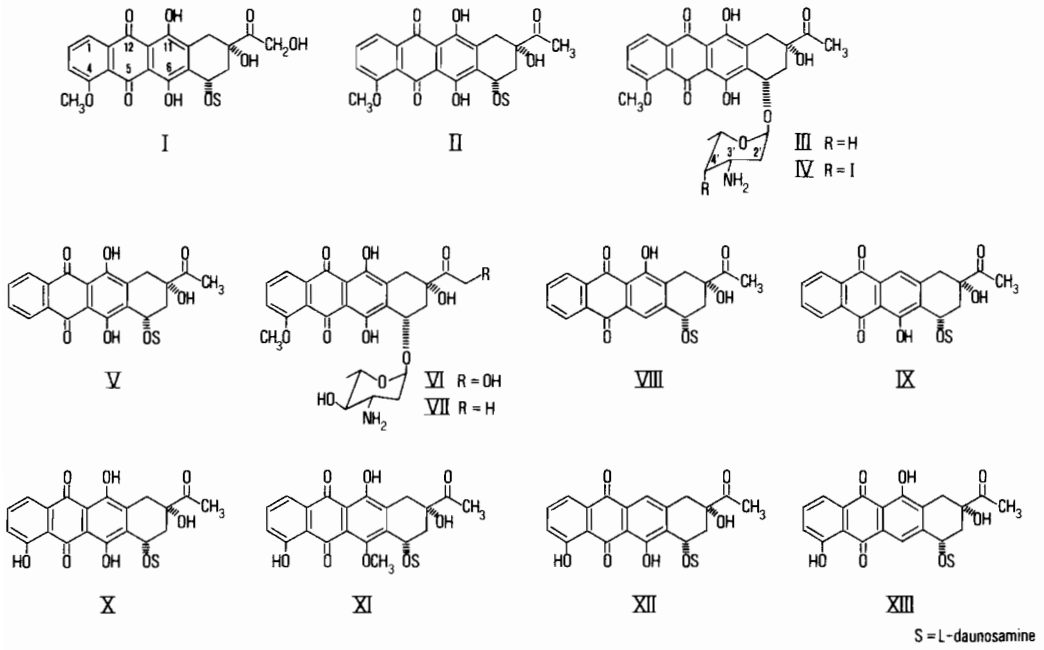
The pH adjustments were made with 0.01 N NaOH or HCl solutions and the values determined with a Gibertini DP 100-NE pH meter at room temperature. A small amount of ethylene glycol was added in order to get glassing solutions for the ESR measurements at −150 °C. The ESR spectra were recorded on a Varian E 109 spectrometer, equipped with a variable temperature accessory, and DPPH was used for the field calibration.

Results

Adriamycin (I), Daunomycin (II), 4'-Deoxydaunomycin (III), 4'-Deoxy-4'-iodo-daunomycin (IV) and 4-Demethoxydaunomycin (V) (Scheme 1)

Although the shape of the ESR spectra of each individual anthracycline/Cu(II) solution is characteristic of tetragonal copper(II) complexes, they show broadened features and no hyperfine interaction in the parallel region of resonances (Fig. 1F). This is due to the presence of spin–spin couplings between different metal centers. The pH value at which the drug–copper complex signals start to coexist with the free Cu(II) hexaqua ion (Fig. 1A) varies with the structure of the anthracycline analogue and might depend upon slight variations of the individual pK_a values of the phenolic hydroxyls.

As already reported [14] the presence of dioxygen favours the formation of the complex in that the onset of complexation takes place at lower pH values



Scheme 1.

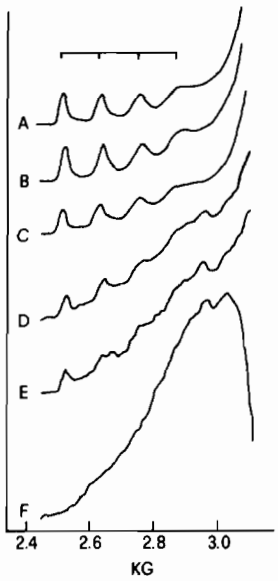


Fig. 1. ESR spectra of 10^{-3} M frozen (−150 °C) aqueous solutions of $CuCl_2$/adriamycin in 1:1 molar ratio at pH: A, 5.0; B, 5.5; C, 6.0; D, 6.3; E, 6.5; F, 7.0. The resonances in the $g_{\|}$ region gradually broaden as the Cu(II)/adriamycin derivative substitutes $Cu(H_2O)_6^{2+}$.

and the effect may be reversed by bubbling N_2 through the solution. The spectrum morphology is fully reversible upon changing the pH of the solution.

4'-Epiadriamycin (VI), 4'-Epidaunomycin (VII), 4-Demethoxy-6-deoxydaunomycin (VIII) and 4-Demethoxy-11-deoxydaunomycin (IX)

The ESR spectra of the Cu(II) complexes of these anthracyclines **(VI–IX)** are similar in that a clearly resolved parallel region was obtained for all of them (Fig. 2). This requires that the metal is a magnetically diluted center in the field of the above mentined ligands. The onset of complex formation is pH and structure dependent and reversibly sensitive to the presence or absence of dioxygen (*vide infra*). The magnetic parameters are reported in Table I.

TABLE I. ESR Parameters of Cu(II) Complexes

Compound	$g_{\|}$	$g_{\perp}$	$A_{\|}$ (G)
$Cu(H_2O)_6^{2+}$	2.39	2.076	120
$Cu(4'\text{-epiadriamycin})_2^{2+}$	2.24	2.050	180
$Cu(4'\text{-epidaunomycin})_2^{2+}$	2.24	2.050	180
Cu(4-demethoxy-6-deoxydaunomycin)$_2$	2.23	2.050	180

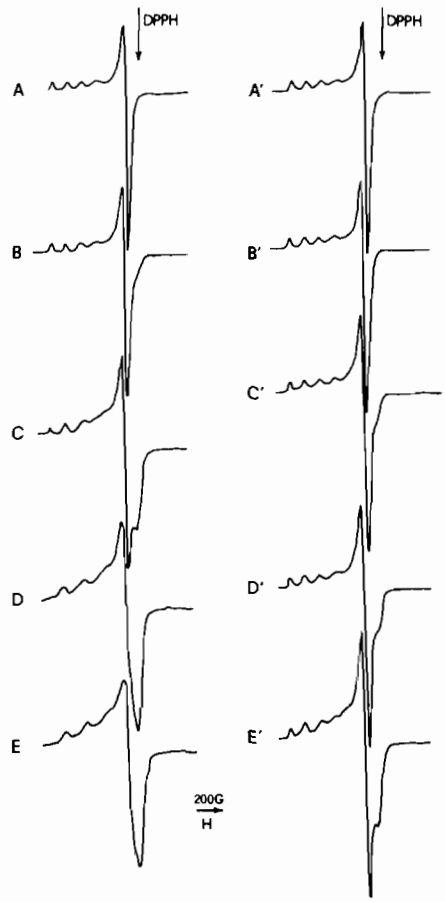


Fig. 2. ESR spectra of 10^{-3} M frozen (−150 °C) aqueous solutions of $CuCl_2$/4-demethoxy-6-deoxydaunomycin in 1:1 molar ratio at pH: A, 5.0; B, 5.5; C, 6.0; D, 6.3; E, 6.5; F, 7.0. Primed letters indicate the spectra at the above pH values of the corresponding deaerated solutions.

Carminomycin (X) 6-O-Methylcarminomycin (XI), 11-Deoxycarminomycin (XII) and 6-Deoxycarminomycin (XIII)

The presence on C(4) of a hydroxyl group provides a new **(XI, XII, XIII)** or a further **(X)** complexation site defined by the C(4)–OH, C(5)=O functionalities. As a result the ESR spectra reveal a mixture of the possible expected complexes. The spectral features are generally broadened and/or superimposed. An example of overlapping in the parallel region of resonances is seen in the spectrum of **XII** (Fig. 3 E,E') where the perpendicular resonances of $Cu(H_2O)_6^{2+}$ and of two possible Cu derivatives of **XII** are clearly distinguishable.

Discussion

Although the complexation properties of anthracycline metal ions were cited among the series of cursory observations made by Calendi *et al.* [9]

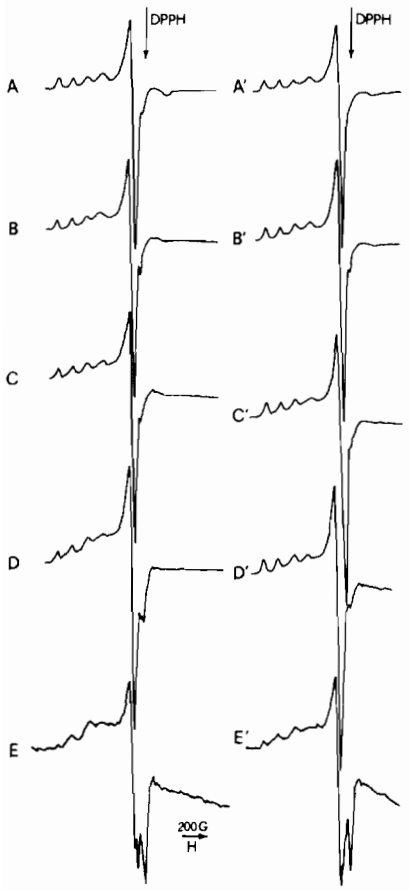


Fig. 3. ESR spectra of 10^{-3} M frozen (−150 °C) aqueous solutions of $CuCl_2$/11-deoxycarminomycin in 1:1 molar ratio at pH: A, 5.0; B, 5.5; C, 6.0; D, 6.3; E, 6.5; F, 7.0. Primed letters indicate the spectra at the above pH values of the corresponding deaerated solutions.

almost 20 years ago, no systematic analysis of the structure-dependent chelating behavior was undertaken. Our study has examined such behavior for a host of ligand molecules, typifying the major modifications of the basic anthraquinone structure, *i.e.*, shifting, removal and/or blocking of the phenolic hydroxyl groups (C(6)–OH, C(11)–OH); and of the daunosamine sugar moiety, *i.e.*, chirality or substitution changes at C′(4), C′(3) and/or removal of the hydroxyl and amino group.

The copper(II) ion behaves as a powerful probe for identifying and detecting the above structural modifications in a manner very similar to the one described for the interaction of biomolecules with metal ions. Three classes of anthracyclines can be identified: (a) adria-like; (b) 4′-epiadria-like; and (c) border line-type.

The first class (a) is made up of anthracycline analogues (Scheme 1: **I, II, III, IV, V**) having broadened ESR spectral features. This is attributed to the formation of polynuclear Cu(II) complexes, made possible by the metal chelation of both the anthracycline hydroxyanthraquinone (C=O, C–OH) groups. Class (b) forms mononuclear copper(II) chelates with a well defined and resolved parallel region of the ESR spectrum. Finally, the border line-type class (c) gives a mixture of mono and polynuclear complexes as a further chelating group is present at C(4).

We define mononuclear complexes as those characterized by a structure in which Cu(II) does not experience any spin–spin exchange interaction with another nearby paramagnetic metal ion [14]. The structural requirements leading to such a structure are: lack of a second coordinating OH at either C(6) or C(11); blocking of one of the phenolic OHs, as e.g., a methyl ether; and inverted chirality (with respect to adriamycin) at C′(4).

The above mentioned behavior of different anthracycline derivatives and the axial symmetry of the magnetic tensors suggest the most probable stoichiometry is $Cu(II)(drug)_2$, where one of the anthraquinone moieties is coordinated to copper in a square-planar geometry. Since the spectra of the adria-like Cu(II) complexes are, though unresolved, very similar to those of the 4′-epiadria-like Cu(II) derivatives, the metal chelation by two anthraquinone moieties is confirmed for the polymeric Cu(II) complexes.

The hydroxyanthraquinone causes a strong field around Cu(II); indeed the size of $g_\parallel$ and $A_\parallel$ values, usually taken as an indication of the field strength in Cu(II) compounds, are more reminiscent of nitrogen-containing Cu(II) chromophores than of tetraoxo Cu(II) ligands [15]. The ground state electronic configuration of all the anthracycline copper(II) complexes here reported is $(d_{xz,yz})^4(d_{xy})^2(d_{z^2})^2$-$(d_{x^2-y^2})$, with the unpaired electron residing in the $d_{x^2-y^2}$ orbital. This implies that the metal ligand bond is essentially σ in character. However the anomalous strong field interaction if compared with that of other CuO_4 chromophores suggests that a fair degree of back donation from the filled $d\pi_M$ ($d_{xz,yz}$) metal orbitals to the π_L^* anthracycline orbitals should be operative. In addition, we have indirect evidence of π metal–ligand interaction. It was indeed observed that the presence of dioxygen favors the formation of the mononuclear complexes, while having a slight influence on the polymeric structures.

Oxygen generally acts as a σ-donor and π-acceptor ligand, and the metal–oxygen bond strength is a function of both σ and π contributions; thus if dioxygen lies in the same metal field as other π-acceptor molecules, competition would be expected between the two ligands for the metal electrons.

We would like to respectively suggest that the interactions (i) and (ii) are at work in the polymeric

(i) $\pi_L^* \leftarrow d\pi_M \rightarrow \pi_L^*$

(ii) $\pi_{O_2}{}^* \leftarrow d\pi_M \rightarrow \pi_L{}^*$

complexes ((i) adria-like class) and in the monomeric ones ((ii) 4′-epiadria-like class. Both interaction schemes lead to a removal of $d\pi$ electrons from the metal toward the ligands, but in the case of the adria-like class compounds (i) the copper is more actively engaged in the back donation to the anthracycline and to a less extent to the oxygen.

In case (ii), though no difference in the oxidation state of copper was observed, and no reduced oxygen species such as $O_2^{\cdot-}$ were detected as proof of one-electron transfer from Cu(II) to O_2, dioxygen is probably bound in a loose transient fashion to the metal center. This interaction recalls electrons from the metal which should become a stronger acid and in turn induce an easier dissociation of the phenolic OH groups.

Finally, the evidence of the π metal–ligand interaction confirms the planar geometry of the CuO_4 chromophore.

Concluding Remarks

Copper(II) is shown to be a useful probe to reveal:

(a) The basic properties of the hydroxy-anthraquinone moiety that depend on the ring substituting groups (Short Range Interaction) or the chirality of the C′(4) center on the sugar (Medium Range Interaction) in this class of antitumor drugs.

(b) The ability of a metal center to transfer electrons to molecular oxygen; the ease of this transfer depends on the π-donor properties and ultimately on the anthracycline structure.

At present it is not known how the metal ion complexation affects and/or interferes with the various mechanisms of action suggested for this class of drugs. It is likely that the main perturbation is at level of the redox properties of the anthracycline ligand and therefore the enzymatic reductive activation of these drugs might be drastically affected.

The electrochemical behavior of the complexes is presently being studied and will be published elsewhere.

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