Chelation of Copper(II) Ions by Doxorubicin and 4′-Epidoxorubicin: ESR Evidence for a New Complex at High Anthracycline/Copper Molar Ratios

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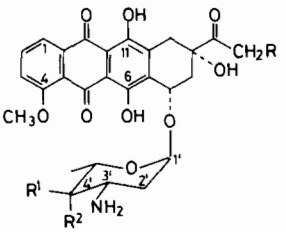
Abstract

Doxorubicin and 4′-epidoxorubicin form chelate complexes with Cu(II), the structures of which are dependent on the drug-to-metal ratio *r*. The complex stoichiometry is defined by the structure of the drug, the pH and the *r* values. The cupric ion is able to evidence minor structural differences (doxorubicin *versus* 4′-epidoxorubicin), the substitution pattern of the antraquinone moiety and the self-association of the anthracycline ligand.

Introduction

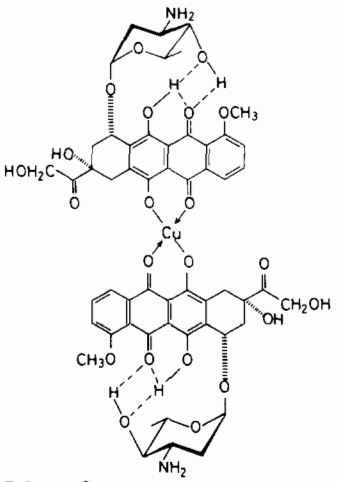
An understanding of the interaction between metal ions and antitumor anthracyclines is deemed to be important, as many recent studies have indicated that the chelated forms of adriamycin (doxorubicin) may contribute to its biochemical activity. Raman resonance spectroscopy has recently been used by Garnier-Suillerot's group [1, 2] to define the structural features of the Cu(II) and Fe(III)–doxorubicin chelates. Sugioka and Nakano [3] have shown that the phospholipid peroxidation is induced by a ternary Fe(II)–ADP–adriamycin complex. DNA damage and oxidative destruction of erythrocyte ghost membranes can be caused by the ferric doxorubicin complex [4, 5]. Although the biochemical effects of the metal ions–anthracycline chelates have been shown, there is a dearth of detailed structural parameters for these complexes.

In a previous communication [6] we have described the different complexation behavior exhibited by two anthracyclines differing in the chirality of the daunosamine sugar C_4' center, *i.e.* doxorubicin (**I**) and 4′-epidoxorubicin (**II**) (Scheme 1). At a drug-to-metal ratio $r = 1$, **I** forms a Cu(II) metal complex for which the observed metal–metal spin exchange interactions require a polynuclear structure, whereas **II** gives a square planar tetracoordinate mononuclear complex having a 2:1 drug/metal stoichiometry: [Cu(II)(4′-epidoxorubicin)$_2$] (Scheme 2).



R = OH ; R¹ = H ; R² = OH doxorubicin
R = OH ; R¹ = OH; R² = H 4′epi-doxorubicin

Scheme 1.



Scheme 2.

We have now gathered ESR evidence relative to a new type of Cu(II) metal complex that forms at high drug-to-metal ratios and which was not shown in previous spectrophotometric titration studies [1].

Experimental

Aqueous solutions obtained by mixing $CuCl_2$ (10^{-3} M) and anthracyclines (10^{-3} M) were brought

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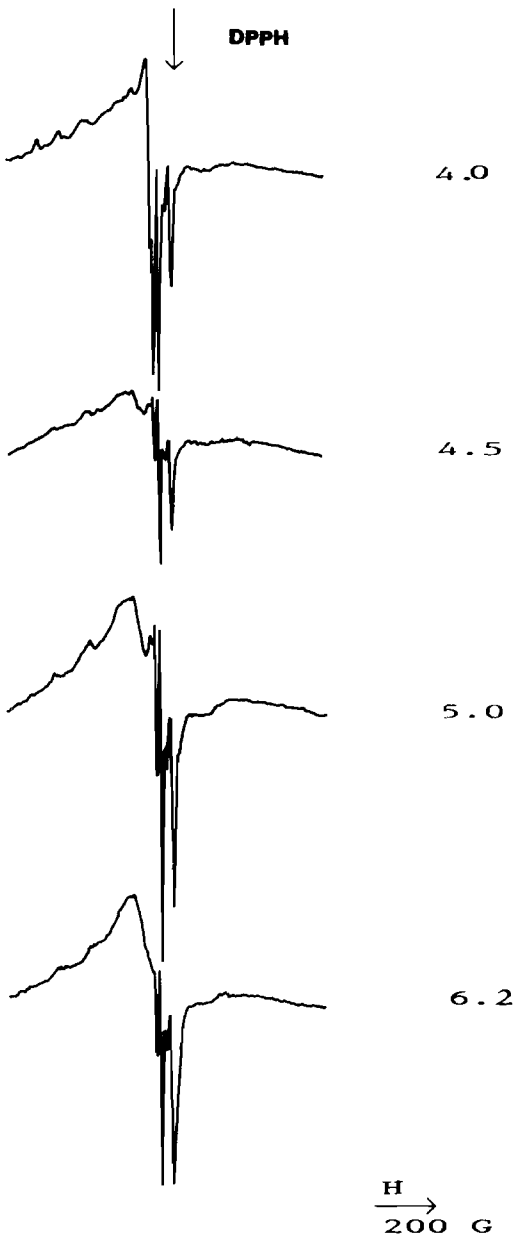

Fig. 1. Dependence of ESR spectra of Cu(II)–4′-epidoxorubicin on pH for r = 10; the pH values are indicated.

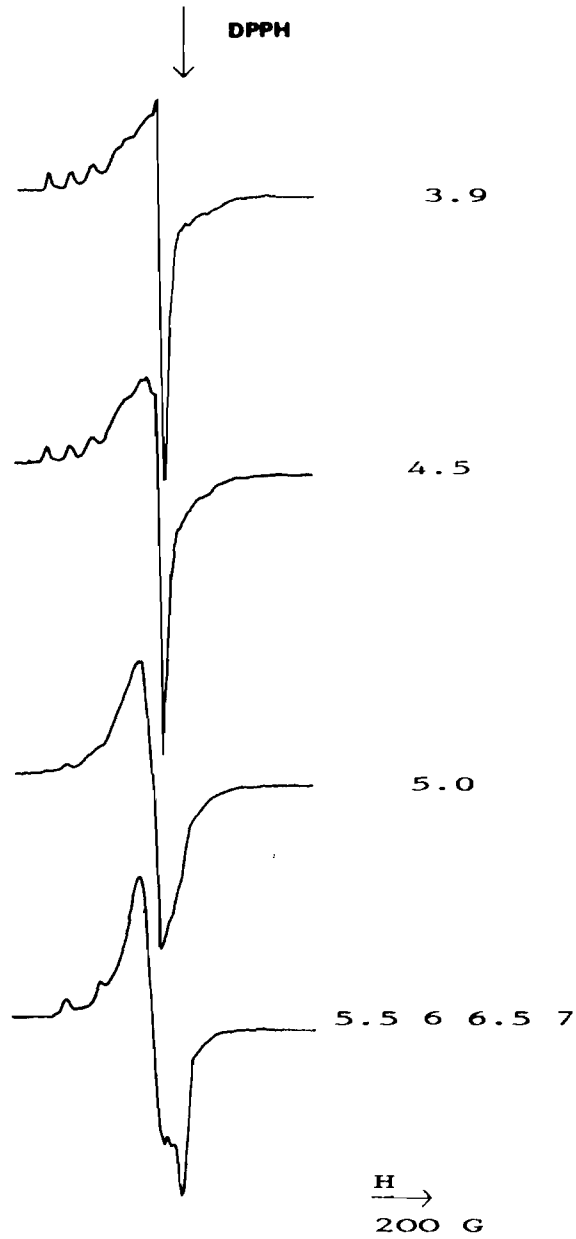

Fig. 2. Dependence of ESR spectra of Cu(II)–4′-epidoxorubicin on pH for r = 2; the pH values are indicated.

to the desired pH value by addition of 10^{-2} M solutions of either HCl or NaOH. A few drops of ethylene glycol were added in order to have glassing solutions at −150 °C. The samples were transferred to an ESR quartz cylindrical cell, which was purged with prepurified N_2 whenever necessary, stoppered and brought to liquid N_2 temperature inside the cavity of a Varian E-109 spectrometer equipped with an automatic temperature controller. DPPH was used to calibrate the field.

Results

Cu(II)–4′-Epidoxorubicin

The ESR spectra of glassing aqueous solutions of 4′-epidoxorubicin and Cu(II) with r = 10 were recorded at different pH values (Fig. 1). At pH = 4 the $Cu(II)(H_2O)_6$ aquo-ion spectrum ($g_\parallel$ = 2.39, $A_\parallel$ = 120 G, $g_\perp$ = 2.08) partially overlaps that attributable to a new paramagnetic species ($g_\parallel$ = 2.28, $A_\parallel$ = 176 G, $g_\perp$ = 2.059, $A_\perp$ = 32 G). The ESR param-

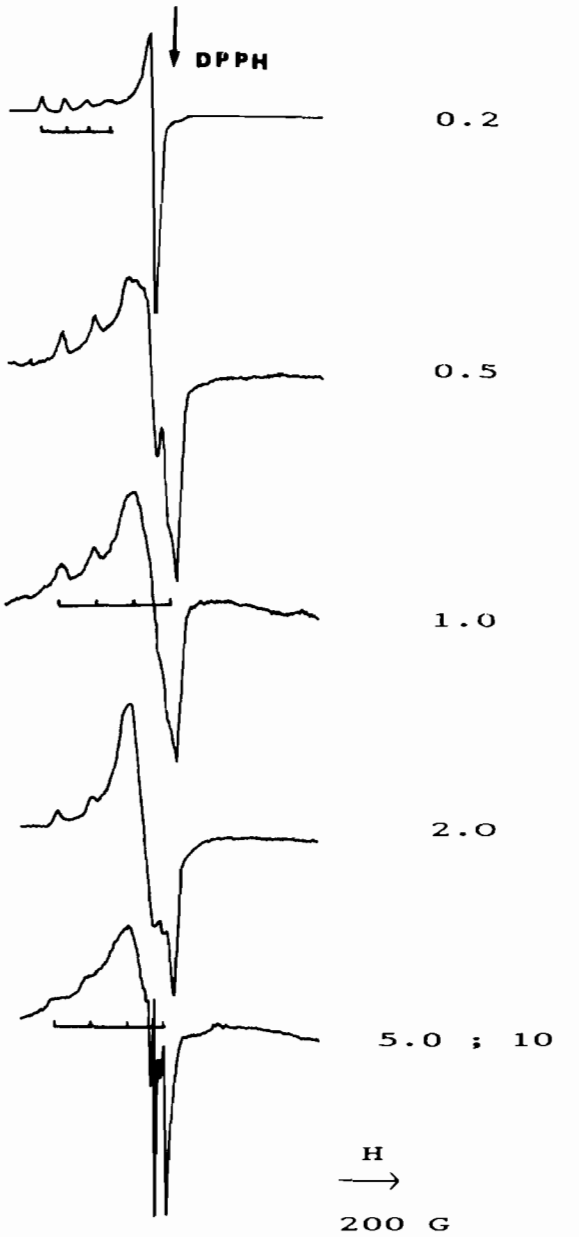

Fig. 3. Dependence of ESR spectra of Cu(II)–4′-epidoxorubicin on r at pH = 6.5.

eters of the latter species are different from those of the previously identified mononuclear complex [Cu(II)(4′-epidoxorubicin)$_2$] and the $A_\perp$ is clearly resolved.

By increasing the pH value, this new species becomes predominant until it is the only one present at pH values $\geqslant$5. It is worth noting that at the drug-to-metal ratio r = 1 pH 6 marked the onset of the appearance of the copper mononuclear complex. No major differences are observed for systems characterized by r = 5 and r = 10 at the various pH values used. However, by lowering the molar ratio r to 2,

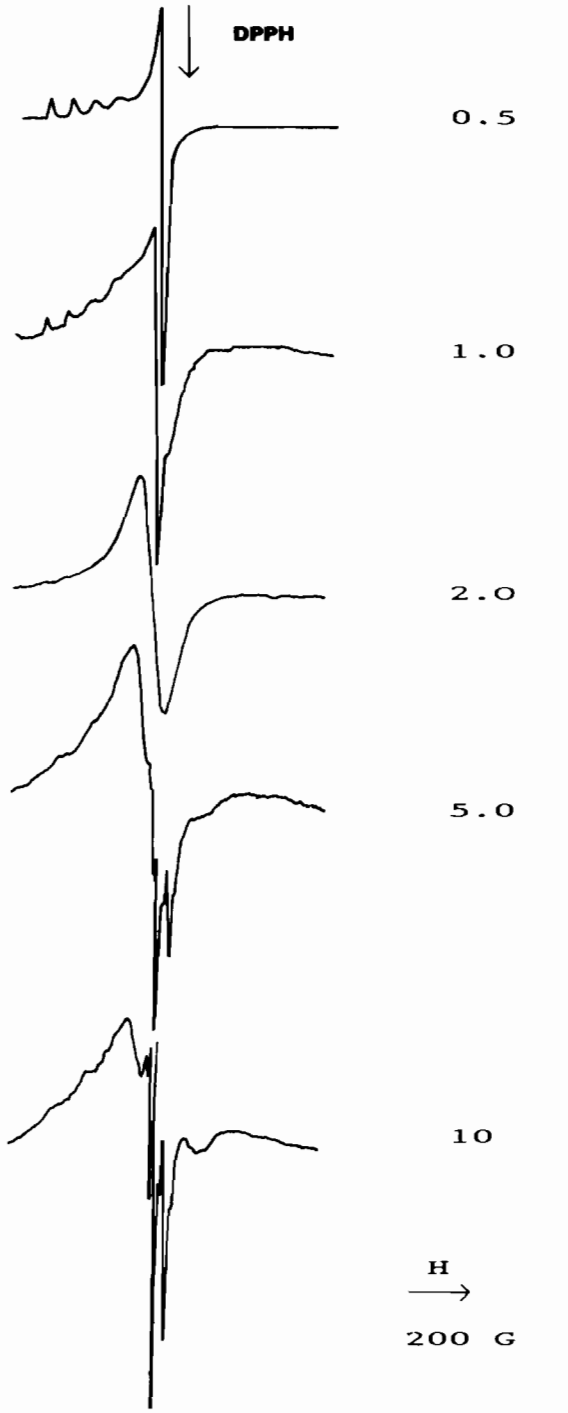

Fig. 4. Dependence of ESR spectra of Cu(II)–doxorubicin on r at pH = 6.5.

the hexaquo-ion Cu(II)$(H_2O)_6$ is present up to pH = 4, then a broadening of the resonances becomes relevant between pH 4 and 5, and by pH 5.5 the spectrum due to the mononuclear [Cu(II)(4′-epidoxorubicin)$_2$] is clearly distinguishable (Fig. 2).

When we operate in the presence of an excess of Cu(II), e.g. $r = 0.2$, the only species observed is $Cu(II)(H_2O)_6$ and at pH $\geqslant 6$ precipitation of copper hydroxides occurs. Figure 3 shows the r dependence of the ESR spectra at a given pH value.

Cu(II)–Doxorubicin

We chose the pH value 6.5 for studying the influence of r values on the complexation pattern (Fig. 4). When operating with an excess of doxorubicin, e.g. $r = 10$, the same paramagnetic species observed in the case of 4′-epidoxorubicin was found. However, the results we obtain for $r = 2$ are at variance with those observed with the ligand 4′-epidoxorubicin in that a magnetically coupled species is required in order to explain the broad spectrum features. For $r = 1$, a small amount of anthracycline/Cu(II) complex is present besides the free copper hydrate ion, the latter species becoming the only one present at $r < 1$.

Discussion

The results we obtain at different drug-to-metal ratios confirm our previous observations for $r = 1$ and also allow us to demonstrate the power of the ESR technique in unravelling the metal complexation behavior of the anthracycline ligand. The new paramagnetic species that we have identified is, as the ESR parameters suggest, still a mononuclear copper–anthracycline complex, but the immediate electronic environment of the ligand is different.

The ESR parameter values, *i.e.* a larger $g_\parallel$ and a lower $A_\parallel$ with respect to the $g_\parallel$ and $A_\parallel$ values reported for [Cu(II)(4′-epidoxorubicin)$_2$] [6], are indicative of a more positively charged complex [7]. We would like to suggest that a different type of interaction might take place at high drug-to-metal ratios that can result in a change of the complex charge. As at the concentration value we operate at (10^{-3} M) the anthracycline molecule is 'stacked up' and forms dimers, tetramers, and oligomers [8] the C_6–OH and C_{11}–OH pK_a values must be different from those observed for more dilute solutions. Recent work by Garnier-Suillerot *et al.* [1] seems to support our suggestion as it is reported that at a concentration of 10^{-5} M the phenolic pK_a values are lower (more acidic OH) than those observed at 10^{-3} M.

The acidity constant difference might then be reflected in the structural features of the complex formed at $r = 10$. When r decreases, the copper ions present in a relatively higher concentration induce an 'unstacking' process and the ligand approaches the monomeric structure. At high r values the copper ion is somewhat diluted within the ligand self-associated structure and the ESR spectrum calls for a mononuclear copper complex, where the metal ion experiences a 'stacked up' ligand environment (Fig. 5).

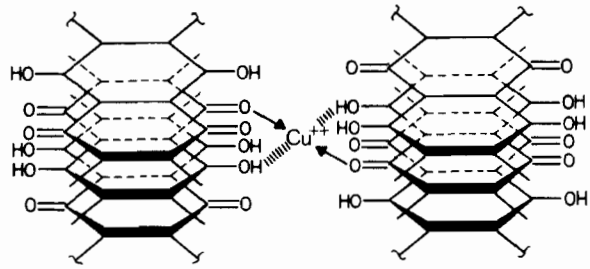

Fig. 5. Scheme to show structure of Cu(II)–4′-epidoxorubicin and doxorubicin for $r = 10$.

It is interesting to consider the different behavior displayed by doxorubicin at $r = 2$ with respect to that of 4′-epidoxorubicin at the same r value. The latter molecule yields a complex in which Cu(II) is magnetically isolated (diluted), while the doxorubicin–Cu spectrum is severely broadened because of spin–spin interactions among paramagnetic centers. This can be taken to mean that Cu(II) ions induce disaggregation of the 'stacked up' anthracycline molecules, *i.e.* 4′-epidoxorubicin and doxorubicin.

However, while this transition leads in the case of 4′-epidoxorubicin to a mononuclear complex of the type already described [6], for doxorubicin a polymeric complex is observed in which the Cu(II) ion spins are exchanged. We find no case where doxorubicin in the range of r values considered is able to form a mononuclear complex beside the 'stacked up' one. Finally, we would like to point out that the formation of the 'stacked up' mononuclear Cu(II) complex does not seem to depend upon the presence of dioxygen and this is at variance with the oxygen requirement displayed by the 'unstacked' mononuclear complexes [6].

Conclusions

It is clear that Cu(II) behaves as a probe for detecting: (i) the strength of the interaction between the paramagnetic ion and the acidic functionalities (phenolic OH) of the anthracycline molecule (Short Range Interactions); (ii) a change of chirality and the different complexation that ensues (Medium Range Interactions); (iii) intermolecular Van der Waals type of interactions like the stacking and unstacking of the ligands (Long Range Interactions).

The definition of the modifiable chelation properties of anthracyclines which rest upon drug-to-metal ratio, pH and chromophore substitution pattern can be helpful in identifying the conditions existing inside the cellular systems and that define which of the above interactions will prevail.

Acknowledgement

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