Cu(II)–5-Iminodaunorubicin Complex. A Circular Dichroism Study

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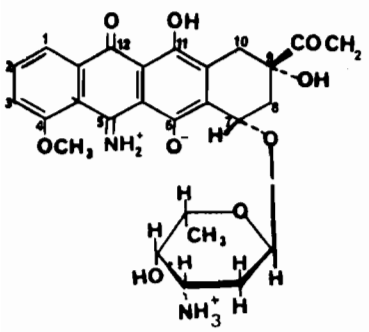
In the course of our studies on the interactions of anthracycline type antitumor compounds with metal ions, undertaken as a way to get less cardiotoxic semi-synthetic derivatives, we have shown that anthracyclines can complex various metal ions and that the site of complexation depends on the nature of the metal ions [1–6]. Data in agreement with this have been reported by other research groups [7–10]. Thus Fe(III) binds to anthracycline at a site involving the C_{12}-carbonyl and the C_{11}-phenolate oxygen forming a six-membered chelate ring [2–4, 7]. The 11-dihydroxyadriamycin and aclacinomycin, which also lack the hydroxyl group on C_{11}, cannot bind Fe(III) [5, 8]. Pd(II) complexation to anthracycline involves the same site of complexation and, in addition, the amino group of the sugar [5]. The case of Cu(II) complexation to anthracycline is less systematic and it seems that Cu(II) can bind either at the C_{11}–C_{12} and/or C_5–C_6 oxygen sites [1, 10].

In this context, 5-iminodaunorubicin (5-ID) (Scheme 1) is an interesting derivative as the two metal binding sites are chemically different: the binding site at C_{11}–C_{12} involves carbonyl and phenolate oxygen whereas that at C_5–C_6 involves imine nitrogen and phenolate oxygen.



Scheme 1.

In this communication we present absorption and circular dichroism (CD) data showing that Cu(II) forms a complex with 5-ID in which two molecules of drug are bound to one Cu(II) ion. The site of complexation involves the C_5-imino nitrogen and the C_6-phenolate oxygen. Moreover, depending on the conditions of preparation, a stacking of the complex molecules can be obtained.

5-ID was provided by Laboratoire Rhône-Poulenc. The solvent used throughout the experiments was either water or Hepes buffer (0.05 M, pH 7.4). The addition of Cu(II) to 5-ID in Hepes buffer solution gave rise to the formation of a complex. This was attested by the decrease of the absorption band at 550 nm as well as the appearance of a CD spectrum of the couplet type with a negative band at 520 nm and a positive one at 580 nm (Fig. 1). In order to determine the stoichiometry of the complex, increasing quantities of Cu(II) were added to a 5-ID solution at molar ratios of Cu(II) to 5-ID varying from 0:1 to 1:1. The plots of ϵ at 550 nm and $\Delta\epsilon$ at 520 levelled off at molar ratios of Cu(II) to 5-ID higher than 1:2, indicating that a 1:2 Cu(II)–5-ID complex was formed. The addition of 50% ethanol to a Cu(II):5-ID 1:2 solution gave rise to a modification of the CD signal: the couplet type signal

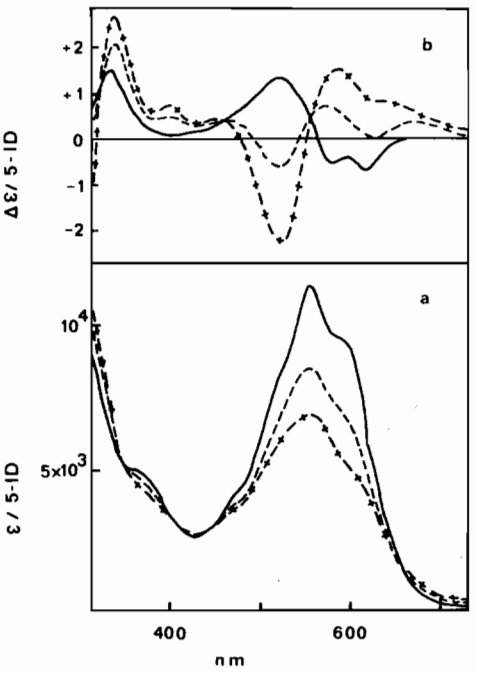


Fig. 1. Absorption (a) and circular dichroism (b) spectra of 5-ID in the presence of various amounts of Cu(II). Experimental conditions were as follows: [5-ID] = 160 μM in 0.05 M Hepes buffer, pH 7.4; the molar ratio of Cu(II) to 5-ID was 0 (——), 0.25 (– – –), 0.5 (– + –).

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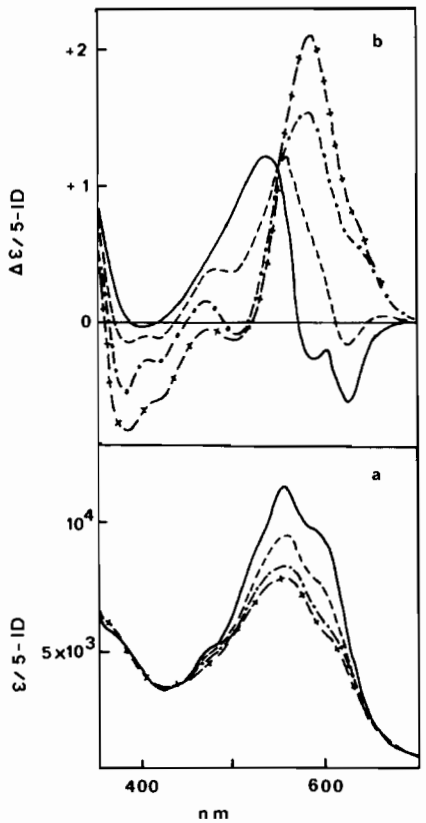

Fig. 2. Absorption (a) and circular dichroism (b) spectra of the Cu(II):5-ID 1:2 system at various pH values. Experimental conditions were as follows: [5-ID] = 130 mM in aqueous solution; pH 3.5 (——), 4.5) (– – –), 5.5 (– · –), 6 (– + –).

disappeared and was replaced by a positive band at 580 nm.

In order to determine the pK of formation of the complex, spectrophotometric titration of the Cu(II): 5-ID 1:2 system in aqueous solution was performed. When the pH was increased from 3.5 to 6.0 the absorption spectrum evolved from that of the free drug to that of the complex previously obtained. The CD spectrum evolved also (Fig. 2) and when the complex was fully formed, at pH 5.5, it only exhibited one positive band, analogous to that previously observed by addition of 50% ethanol to the Cu:5-ID 1:2 complex in Hepes buffer. The pK of formation was equal to 4.5.

The aglycone part of the 5-ID molecule has two sites of coordination available for Cu(II) ions: one at C_{11}–C_{12} involving two oxygen atoms, the other one at C_5–C_6 involving one nitrogen and one oxygen atom. It is known that shift of the absorption band of anthracycline is observed only through deprotonation of the hydroxyl group on C_6 or C_{11} which can occur either through a rise in pH or through metal complexation. This led us to propose that in free 5-ID, at acidic pH and up to pH 9, the proton on the C_6-hydroxyl group lies in fact on the C_5-imino group [4]. On the other hand, we have shown that complexation of Fe(III) to 5-ID occurs at the C_{11}–C_{12} oxygen atoms and is characterized by a shift of the absorption band to higher wavelength [4]. In the present study, the observation that complexation of Cu(II) to 5-ID does not give rise to spectral shift can be taken as an indication that the complexation of Cu(II) occurs at the site involving a hydroxyl group which is already deprotonated, *i.e.* at C_5-C_6. We thus propose that in this complex, Cu(II) ion is bound to two molecules of 5-ID through the C_5-imino nitrogen and the C_6-phenolate oxygen, thus forming a six-membered chelate ring.

It is well known that, in aqueous solution, due to hydrogen bonding, a stacking of the planar molecules of the drug occurs. This type of interaction appears as the concentration of the drug increases [11]. However, this stacking can be promoted by the presence of Cu(II) ions and this is the case for the 1:1 and 1:2 Cu:aclacinomycin species, or prevented by the presence of Cu(II) and this is the case for the 1:2 Cu:carminomycin species [6]. This type of interaction is characterized by a CD signal of the couplet type. This type of interaction, together with the CD signal of the couplet type, disappears in 50% aqueous ethanol solution.

Our data show that, depending on the preparation used, a stacking of the complex molecules can occur. Thus when the complex is prepared by addition of Cu(II) to 5-ID in Hepes buffer a stacking of the molecules takes place. However, when the complex is prepared by increasing the pH of the Cu:5-ID 1:2 system in aqueous solution the same complex is obtained but no stacking is detected.

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