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MONONUCLEAR AND BINUCLEAR Co(III)-DIOXYGEN ADDUCTS OF BLEOMYCIN. CIRCULAR DICHROISM AND ELECTRON PARAMAGNETIC RESONANCE STUDIES.

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SUMMARY : Co(II) interacts with bleomycin in aqueous solution, in the presence of air, to give a short lived mononuclear superoxo-Co(III) complex (I) identified previously, by Sugiura, by electron paramagnetic resonance measurements. This complex rapidly releases O_2 to yield the dinuclear μ-peroxo-Co(III) complex (II), but is stabilized by the presence of DNA yielding a new superoxo long lived species (I'). The absorption and circular dichroism spectra of the three species (I, I', II) have been characterized.

INTRODUCTION : The bleomycins (BLM) are a family of glycopeptide antibiotics which are clinically prescribed for certains tumors (1). The effects of different concentrations of metal free BLM and its Cu(II), Fe(II), Zn(II) and Co(II)-complexes upon Ehrlich cells in culture was studied by Petering et al. (2). They showed that with the exception of the Co(II)-complex all of the compounds tested, including free BLM inhibited cell proliferation.

In recent years, the interaction of Co(II) with BLM has been a subject of increasing interest (3-6); Sugiura, in particular, showed via EPR measurements that a low spin cobalt complex, presumably BLM-Co(III)-O_2^-, is formed by addition of Co(II) to an aqueous solution

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Abbreviations used : BLM, bleomycin; DNA, deoxyribonucleic acid; CD, circular dichroism; EPR, electron paramagnetic resonance.

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of BLM (3). However, Vos et al. (5,6) were able to demonstrate that under the conditions studied by Sugiura there is, in fact, an equilibrium between two complexes.

These observations prompted us to initiate an investigation on the interaction of Co(II) with BLM in order to identify accurately both species by means of spectroscopic techniques (absorption, CD and EPR). In addition , the fact that the Co.BLM complexes show no antibiotic activity strongly suggests some particular structure of these species which hampers the free access to the BLM sites essential to this activity. Accordingly,the elucidation of the structure of the Co.BLM complexes should permit a better understanding of the BLM mode of action.

In this communication we present the results of an investigation on the interaction of Co(II) with BLM at different temperatures (between 0°C and 25°C) using spectroscopic techniques and oxygen uptake measurements. We have thus been able to demonstrate that: i) in aqueous solution, in the presence of oxygen, Co(II) reacts with BLM to give the complex identified by Sugiura (hereafter called I). This reaction is fast at room temperature and I dimerizes to give a μ-peroxo complex (hereafter called II); ii) I is stabilized in the presence of DNA yielding a new long lived species (I') with a half life of several days.

EXPERIMENTAL : Purified BLM-A_2 was kindly provided by Laboratoire Roger Bellon. Standard Co(II) solutions were prepared from reagent grade material ($(NH_4)_2 SO_4.6H_2O$). Calf thymus DNA was purchased from Sigma Chemical Company. All others reagents were of the highest quality available and deionized water was used throughout the experiments. Unless otherwise stated samples of oxygenated species were prepared directly by the reaction of the antibiotics and Co(II) salt in aqueous solution (Hepes buffer 0.01 M, pH 7.4) in the open air.

Absorption spectra were recorded on a Cary 219 spectrophotometer; CD spectra on a Jobin Yvon dichrograph model Mark III; EPR spectra on a Varian CSE 109 spectrophotometer at -180°C. The O_2 concentration measurements were performed using a YSI 5331 oxygen probe.

RESULTS AND DISCUSSION : The addition of Co(II) to an aqueous BLM solution in the presence of air gives rise to the rapid formation of

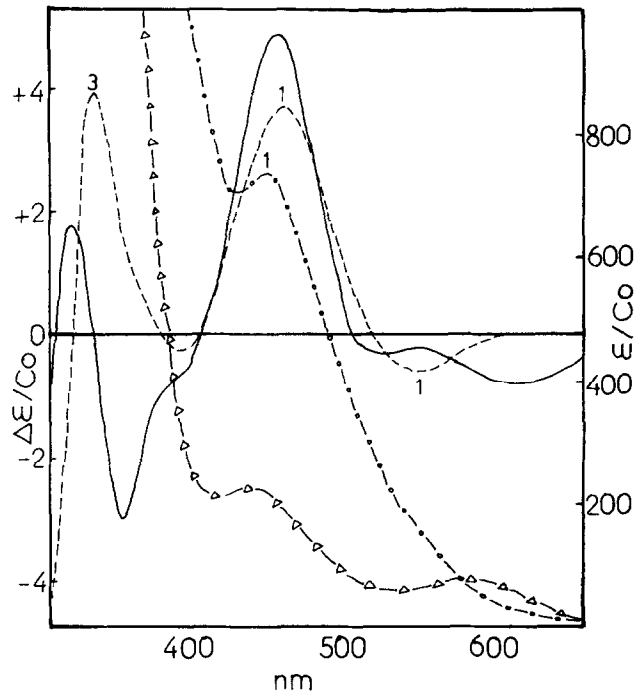

Figure 1 . Absorption spectra of complex I (-•-), complex II (-△-). CD spectra of complex I (- -), complex II (——). Experimental conditions: 200 μM Co(II), 350 μM BLM in 0.01 M Hepes buffer pH 7.4. For complex I: T= 2°C; numbers upon bands indicate the time elapsed after addition of Co(II) to BLM. For complex II: T= 25°C;time about 10 minutes.

a brown complex (I) which evolves to a green complex (II). At room temperature the formation and evolution of I to II is too fast to be followed with conventional techniques. However,we observed that the reaction rate greatly decreases at low temperature. In particular, near 0°C, the kinetic becomes low enough to allow an accurate study of complex I. Nevertheless, data must be collected rapidly as complex I, even at ice temperature , has a fairly short life time (minutes).

The formation of complex I at low temperature is attested by the appearance of one band at 470 nm (ε/ Co = 730 $mol^{-1} cm^{-1}$) in the visible absorption spectrum. In Figure 1 are illustrated the absorption and CD spectra of this complex. Concomitantly one mole of oxygen per mole of

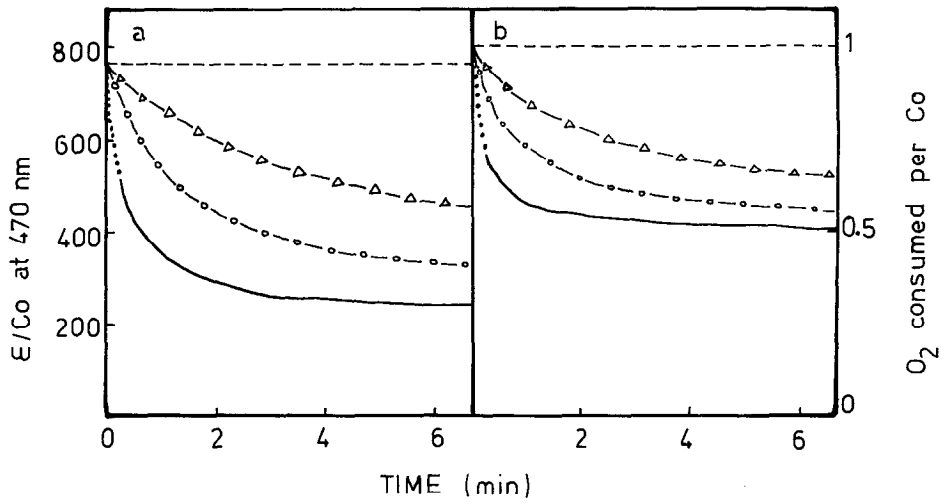

Figure 2 . Time course of reaction I ⟶ II monitored by the absorbancy at 470 nm and the ratio of O_2 consumed per cobalt. Experimental conditions: 200 μM Co(II), 350 μM BLM in 0.01 M Hepes buffer pH 7.4, 25°C (——) ; 14°C (-∘-) ; 7°C (-△-) ; 25°C in presence of DNA 3×10^{-3} M (- -).

Co(II) is consumed (Figure 2b, at t = 0) and an EPR spectrum similar to that observed by Sugiura (3) is obtained (Figure 3a).

The evolution of I to II, on the other hand, is characterized by i) the decrease of the absorption band at 470 nm as time elapses and the presence of an isosbestic point at 580 nm (Figures 1 and 2a), ii) the release of one mole of oxygen per two moles of Co(II) (Figure 2b) , iii) the decrease and final disappearance of the EPR signal. On Figure 1 are illustrated the absorption and CD spectra of complex II.

The values of the half life of complex I at different temperatures are reported in Table I.Similar experiments performed at different Co(II): BLM molar ratios and at several pH show that I and II are both 1:1 complexes fully defined at pH 7.4.

The foregoing results corroborate those of Sugiura (3) for complex I which suggest the formation of a dioxygen adduct of the Co(II)-BLM complex in which the unpaired spin density resides on the dioxygen moiety,i.e. the superoxo-cobaltic complex : BLM-Co(III)-O_2^-.

Table I

Half life of BLM.Co(III).O_2^- complex at different temperatures and in presence of various concentrations of DNA.
Experimental conditions: 200 μM Co(II), 350 μM BLM in Hepes 0.01 M, pH 7.4.

T(°C)	7	14	25	25	25	25
DNA	0	0	0	0.38×10^{-3}M	0.76×10^{-3}M	1.53×10^{-3}M
Half life (seconds)	310	90	20	28	60	560

The absorption spectrum of complex I (Figure 1)is to be compared with that of other mononuclear dioxygen Co(III) complexes which present one strong band at 340 nm ($\varepsilon \sim 5000$) and a weaker one at 490 nm ($\varepsilon \sim 700$) (8,9). They have been assigned respectively to the $\pi^*_h(O_2^-) \rightarrow d\sigma^*$ Co(III) and $d\pi$ Co(III) $\rightarrow \pi^*_v$ (O_2^-) charge transfer transition (9). A quite analogous assignment holds for the two bands of complex I at 340 nm ($\varepsilon = 10^4$, $\Delta\varepsilon = +4$) and at 470 nm ($\varepsilon = 730$, $\Delta\varepsilon = +3.6$).

As can be inferred from Figure 2b, the stoichiometry of complex II (one O_2 per two Co) is that of a dimeric-dioxygen adduct.This, as well as the diamagnetism , strongly suggests for II a μ-peroxo-dicobalt structure, i.e.,$[Co(III)\text{-}BLM]_2O_2^{2-}$ with all electrons paired in the low spin Co(III) and the electrons of dioxygen pairing in the π^* orbital. Thus, the equilibrium I $\rightarrow$II is not unexpected as it is well known that 1:1 superoxo complexes are generally fleeting intermediates on the way to bridging species (7,8). The electronic spectrum of this complex is in agreement with this structure (8). We can assign the two bands at 360 nm ($\varepsilon \sim 3500$, $\Delta\varepsilon = -3$) and at 325 nm ($\varepsilon \sim 7500$, $\Delta\varepsilon = +3.5$) to the $\pi^*_a(O_2^{2-}) \rightarrow d\sigma^*$ Co(III) and $\pi^*_b(O_2^{2-}) \rightarrow d\sigma^*$ Co(III) charge transfer transitions.

The same experiments performed in the presence of DNA show a considerable reduction of the I $\rightarrow$II rate. In table I is reported the half life of

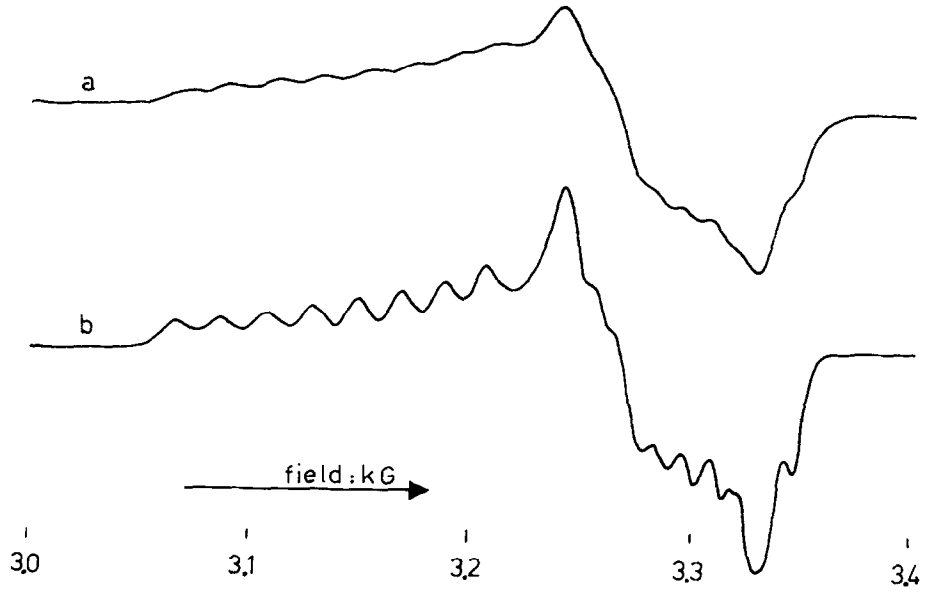

Figure 3 . Curve a : EPR spectrum of complex I ; $g_{//}$ =2.099 , $A_{//}$ =20.0 G
Curve b : EPR spectrum of complex I'; $g_{//}$ =2.100 , $A_{//}$ =20.2 G
Experimental conditions: 200 μM Co(II), 350 μM BLM in 0.01 M Hepes buffer pH 7.4. For complex I mixing of Co(II) solution with BLM solution was performed at 1°C and then immediately frozen to -180°C. Complex I'was formed in presence of DNA 5×10^{-3} M.

complex I in the presence of DNA at several concentrations. In these experiments BLM was always added first to the DNA solution followed by the Co(II) solution. At [DNA] $\geq 3 \times 10^{-3}$M complex I is stable for several days.

Figure 4 shows the absorption and CD spectra of the complex thus obtained (hereafter called I'). The absorption spectrum is quite similar to that of Figure 1 while the CD spectrum is slightly different exhibiting higher dichroism at 390 and 550 nm. On the other hand, the EPR spectrum is much better resolved although $g_{//}$ and $A_{//}$ remain practically unchanged (Figure 3). If one assigns to complex I the structure proposed by Sugiura (3) the ligand trans to the O_2^- must be an amino-nitrogen.As it has been established, superoxo Co(III) complexes are stabilized by a σ donor and/ or a π acceptor ligand trans to O_2^-(10). Hence,one might assume that in complex I' one of the purine or pyrimidine nitrogens of DNA bases super-

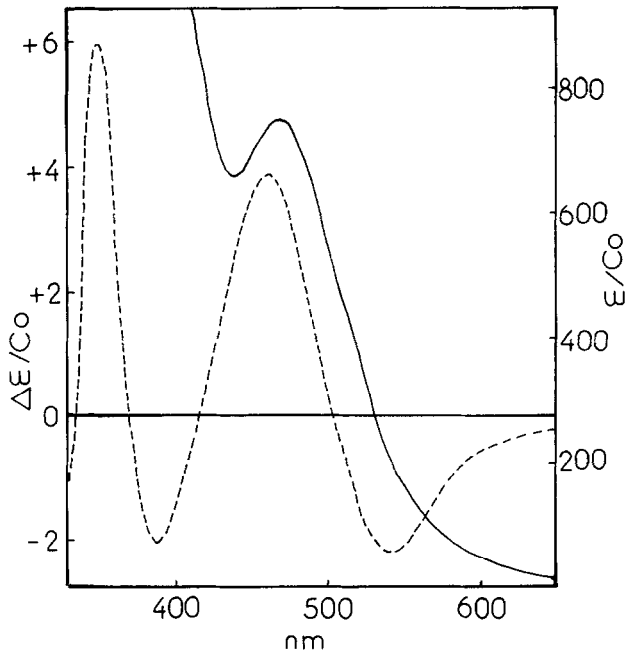

Figure 4 . Absorption (——) and CD (- -) spectra of complex I'. Experimental conditions: 200 μM Co(II) , 350 μM BLM, $5x10^{-3}$ M DNA in Hepes buffer 0.01 M, pH 7.4 at 25°C; time after mixing is about 30 minutes.

sedes the apical NH_2 group of complex I. Preliminary studies on the conformational changes of DNA in the presence of complex I' provides some evidence indicating the involvement of DNA bases in complexation (Garnier, unpublished results). Experiments are currently in progress to elucidate this point.

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