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IRON·BLEOMYCIN·DNA·SYSTEM

EVIDENCE OF A LONG-LIVED BLEOMYCIN·IRON·OXYGEN INTERMEDIATE.

Jean-Paul Albertini[+Δ], Arlette Garnier-Suillerot[+Δ*] and Lucia Tosi[Δ]

Δ Département de Recherches Physiques, Université Pierre et Marie Curie, Laboratoire Associé au C.N.R.S., nº 71, 4 place Jussieu, 75230 Paris Cedex 05

+ U.E.R. de Santé, Médecine et Biologie Humaine, Université Paris XIII, Bobigny.

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SUMMARY : When Fe(II) is added to a bleomycin.DNA mixture in the presence of air a long-lived ($t_{1/2}$ = 45 minutes) EPR silent species (I') is formed ; the circular dichroism and absorption spectra of which have been characterized. This complex slowly decays yielding a ferric complex (III') analogous to the well known low spin Fe(III).BLM species.

INTRODUCTION : Bleomycins are low molecular weight glycopeptide antibiotics used clinically as antitumor agents (1). The precise mechanism by which bleomycins cause cells death is unknown but is thought to be mediated by their interaction with DNA and, in fact, DNA isolated from bleomycin treated cells, is known to be degraded (2,3).

Strand-scission of DNA induced by bleomycins in vitro requieres the presence of Fe(II) and molecular oxygen(4,5). A number of mechanisms have been proposed (6) ; most of them suggest that strand-scission occur between carbon 3' and 4' of the sugar ring and it is generally admitted that this process is initiated by free radicals generated through a ternary BLM.iron. oxygen activated species (6).

The addition of Fe(II) to an aqueous solution of BLM at pH ≃ 7, in the presence of air, yields the well known low spin ferric brown complex :

* Author to whom correspondence should be adressed.

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

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Fe(III).BLM (g=2.45, 2.18, 1.89), in a reaction that takes several seconds. Peisach and coworkers (7,8) have shown that this reaction is the result of three sequential kinetic events : 1) the formation of a short-lived ($t_{1/2}$ = 6 s, 2ºC) EPR silent species (I) which, on grounds of kinetic measurements, they suggested to be a 1.1.1 iron.BLM.oxygen complex (7) ; 2) the decay of this intermediate to "activated BLM",a compound (II) that cleaves DNA, which is a s = 1/2 species (g = 2.26, 2.17, 1.94) ; 3) the slower decay ($t_{1/2}$ = 60 s, 2ºC) of this second species that ultimately yields Fe(III).BLM(III).

In a recent study of the BLM-cobalt system we have shown that the short-lived mononuclear superoxo-Co(III) complex, which is formed when Co(II) is added aerobically to a BLM solution is greatly stabilized in the presence of DNA (9). This observation prompted us to initiate an investigation on the interaction of Fe(II) with BLM in the presence of DNA. Using spectroscopic techniques and oxygen uptake measurements we have been able to demonstrate that : i) the addition of Fe(II) to BLM in the presence of DNA at concentrations equal or higher than 4 x 10^{-3}M yields a rather long-lived species with a half life of about 50 minutes ; this species (hereafter called I') is EPR silent and, at 25ºC, one mole of oxygen per mole of iron is consumed during its formation ; ii) complex I' evolves yielding ultimately an Fe(III).BLM complex (g=2.43, 2.18, 1.89) (hereafter called III').

EXPERIMENTAL : Purified BLM-A_2 was kindly provided by Laboratoire Roger Bellon. Standard Fe(II) solutions were prepared from reagent grade $FeSO_4$. $(NH_4)_2$ $SO_4.6H_2O$. 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 Fe(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 -180ºC. The O_2 concentration measurements were performed using a YSI 5331 oxygen probe.

RESULTS AND DISCUSSION : Iron.BLM.Oxygen interaction. A first set of experiments was performed to determine the O_2 consumption, when Fe(II)

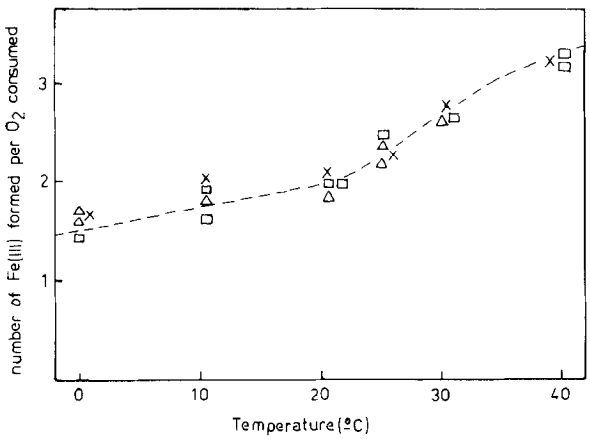

Figure 1. Number of Fe(III) formed per O_2 consumed as a function of temperature. Experimental conditions : Hepes buffer 0.01M pH 7.4 ; 50 µM BLM, 50 µM Fe(II) (Δ) ; 100 µM BLM, 100 µM Fe(II) (□) ; 150 µM BLM, 150 µM Fe(II) (x).

is added to BLM in the absence of DNA. These measurements were done at several temperatures between 0 and 40°C. Whatever the temperature, a rapid uptake of oxygen is observed giving rise to the formation of the well known complex III as indicated by absorption, CD and EPR spectra. The absorption and CD spectra of complex III are illustrated in Figure 3. The CD spectrum exhibits bands at 610 nm (-0.6), 515 nm (+2.05), 395 nm (+2.8), 350 nm (+2.7) and 320 nm (+1.6). The EPR spectrum reveals the presence of 15% of high spin form and 85% of low spin form (g = 2.45, 2.18, 1.89) (10).

This reaction is completed within one minute. It must to be recalled that, owing to the time response of the Clark electrode, we observe, in fact, the overall consumption of O_2 and are not able to distinguish the intermediary steps. At the end of the reaction the concentration of complex III measured by absorption at 385 nm exactly matches the concentration of Fe(II) added to the solution. No release of oxygen is observed after a ten minutes interval.

Figure 1 shows the plot of the number of Fe(III) formed per oxygen consumed as a function of temperature. This ratio is strongly temperature dependent and the pattern of Figure 1 gives clear evidence of, at least,

two reactions showing different dependency. In addition, it must be emphasized that, independently of oxygen uptake and whatever the temperature, the species detected at the end of the reaction is always complex III.

Iron.BLM.DNA.Oxygen interaction. Similar measurements were performed, on the same system, in the presence of DNA at various concentrations. In these experiments the sample was always prepared by mixing the BLM and the DNA solutions before adding the Fe(II) solution. Unless otherwise stated Fe(II) and BLM solutions are 50 μM. The spectra are recorded one minute after mixing at 25ºC.

The O_2 consumption is more rapid than in the absence of DNA and reaches a plateau within 20 seconds, which is approximately the time response of the Clark electrode. We do not observe any release of O_2 during the following ten minutes. For a given temperature (we report here a set of experiments performed at 25ºC) the number of Fe(III) formed per O_2 consumed decreases as the concentration of DNA increases. This is shown in Figure 2 where the number of Fe(III) formed per oxygen consumed is plotted against DNA concentrations. At $[DNA] \geqslant 4 \times 10^{-3}M$, approximately one mole of O_2 is consumed per mole of Fe(III) formed.

In the presence of increasing amounts of DNA the percentage of low spin form decreases following the same trend as the oxygen uptake per mole of Fe(II) (Figure 2). On the other hand, the percentage of high spin form remains practically unchanged. At $[DNA] = 4 \times 10^{-3}M$ the low spin form completely vanishes indicating that an EPR silent species (I') is formed instead. As time elapses the EPR signal of a low spin form III', very similar to that of complex III, slowly reappears. At $[DNA] = 4 \times 10^{-3}M$ the recovery takes about 90 minutes.

Absorption and CD spectra are considerably modified in the presence of increasing concentrations of DNA (Figure 3). In particular, one observes a decrease of the DC band at 515 nm which becomes zero at $[DNA] = 4 \times 10^{-3}M$.

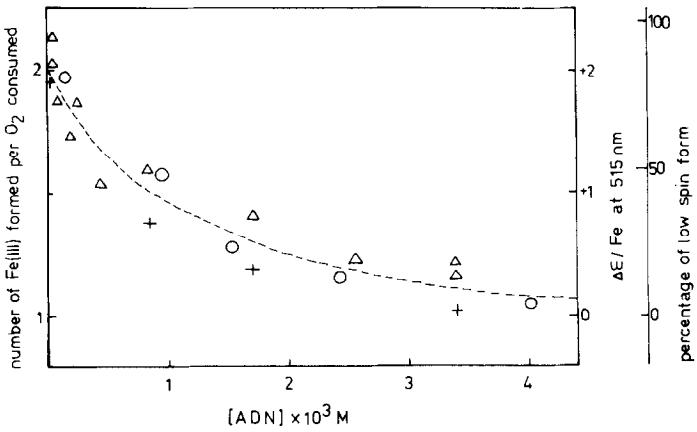

Figure 2. Formation of BLM.Fe(II).O_2 in the presence of DNA.
(Δ) number of Fe(III) formed per mole of O_2 consumed,
(o) molar circular dichroic coefficient at 520 nm,
(+) percentage of low spin form present, plotted against DNA concentrations.
Experimental conditions : Hepes buffer 0.01M pH 7.4 ; 50μM BLM,
50 μM Fe(II) ; t = 25ºC. The various parameters are determined
approximately one minute after mixing.

concomitantly with the vanishing of the low spin EPR signal. As shown in Figure 2 the plots of $\Delta\varepsilon$ at 515 nm against DNA concentration parallel those of oxygen uptake and of percentage of the low spin form of complex III. No further modifications is detected at higher concentrations of DNA.

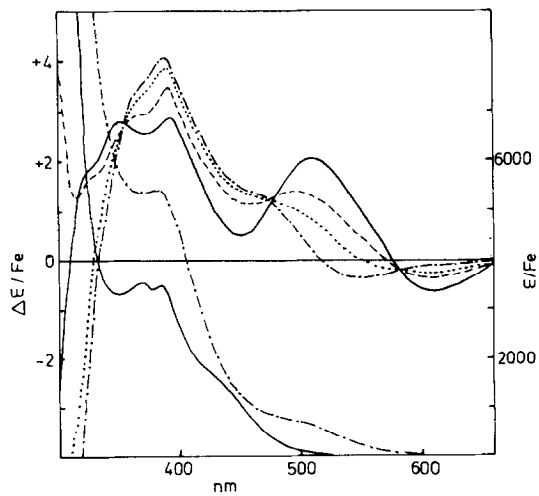

Figure 3. Absorption and CD spectra of complex I' (-.-) and complex III (——).
Experimental conditions : Hepes buffer 0.01M pH 7.4 ; 50 μM BLM,
50 μM Fe(II) and DNA = 0 (——), 0.9 x 10^{-3}M (- - -), 1.5 x 10^{-3}M (-.-) ;
t = 25ºC. Spectra are recorded one minute after mixing.

The CD spectrum of the EPR silent species I' exhibits three bands at 550 nm (-0.4), 470 nm (+1.3), 385 nm (+4.0) and 350 nm (+3.2). Three isodichroic points are observed at 585, 475 and 355 nm.

One may interpret the foregoing results by assigning to complex I' a 1.1.1 iron.BLM.O_2 ternary structure. This assumption is corroborated by the absorption spectrum of this complex, presented in Figure 3, which looks like that obtained by Peisach et al. (7) for the short-lived EPR silent species I. The absorption and CD spectra of complex I' evolve slowly and after about 90 minutes the spectra of complex III' are obtained. These spectra are slightly different from that of complex III suggesting the ligation of DNA to the metal.

From the studies reported here it appears that the presence of DNA stabilizes the short-lived EPR silent species observed by Peisach et al.(7). Stabilization of the BLM.iron oxygen complex may be attributed to the sum of two effects : the binding of a DNA base trans to the oxygen and the building up of a protective pocket around the metal as in hemoproteins. Moreover the half life of the "activated BLM" intermediate (g = 2.26, 2.17, 1.89) (8) seems to become quite short relative to that of complex I' since it is never present in concentration high enough to be detected either by EPR or by CD.

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