Synthetic Cobalt Bleomycin Models as a Photochemical DNA Cleaver

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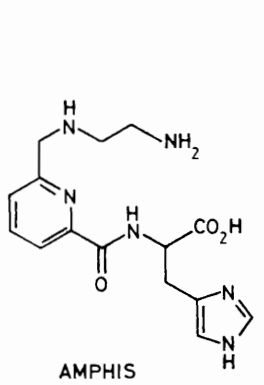
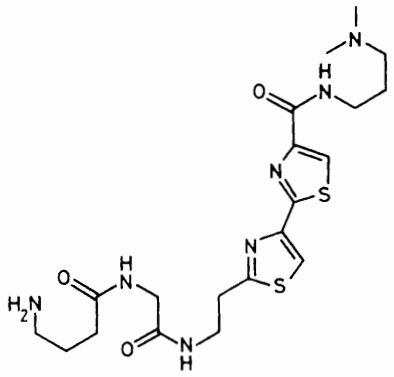
Simplified synthetic models of the bleomycin cobalt complex which contain the AMPHIS {*N*-[6-(2-aminoethylaminomethyl)pyridin-2-ylcarbonyl]histidine} metal-ion binding ligand and DNA binding component have been prepared; the cobalt complexes cleaved DNA upon photoirradiation.

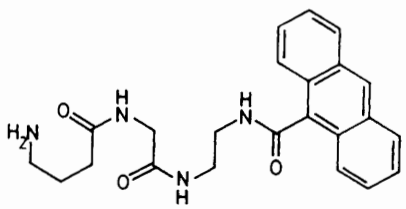
There has been recent growing interest in the design of photochemical DNA cleaving molecules that mediate sequence-selective DNA cleavage upon photoirradiation ('photonucleases').[1] Among the photochemical DNA cleavers so far reported, bleomycin cobalt complex (Co·BLM) is unique in that it efficiently produces alkali-labile sites at 5′-guanine (G)–pyrimidine sequences of double stranded DNA upon photoirradiation at 330—450 nm.[2] We are particularly interested in the synthesis of a simplified Co binding ligand which mimics the Co·BLM-mediated DNA



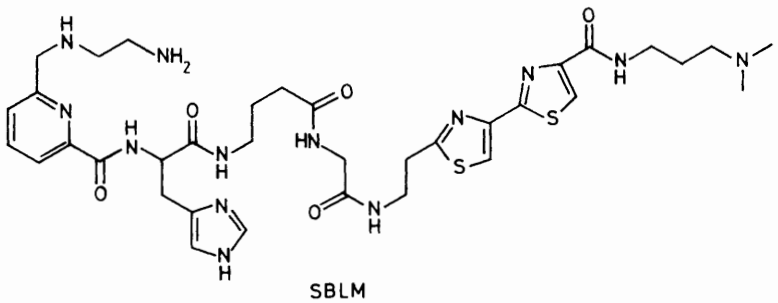
AMPHIS



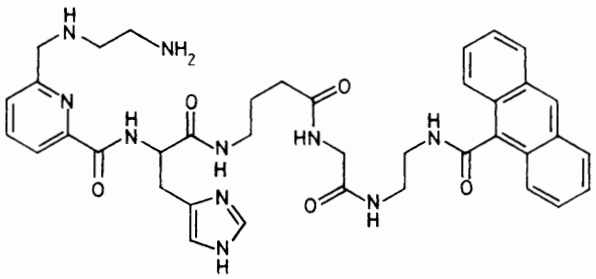
(1)



(2)



SBLM



ABLM

photocleavage and is readily connectable to DNA binding compounds for the design of artificial 'photonuclease.' Several synthetic analogues of the BLM metal-ion binding site have already been reported,[3] and the co-ordination structure of certain Cu complexes[4] and the oxygen activation mechanism by Fe complexes have been actively investigated.[3] However, the Co complex of these simplified BLM analogues and its photochemical behaviour towards DNA have not been studied. We report herein for the first time that when the Co complex of a simplified ligand, AMPHIS, *N*-[6-(2-aminoethylaminomethyl)pyridine-2-ylcarbonyl]histidine,[5] is linked to a DNA binding unit, the molecule can serve as an efficient photochemical DNA cleaver.

AMPHIS prepared as described[5] was condensed [1,1'-carbonyldiimidazole/Et_3N/dimethylformamide (DMF)] with the terminal amino group of (**1**).[6] By removing protecting groups (HBr/AcOH), the crude mixture was converted to the Cu^{II} complex which was further purified by reversed phase h.p.l.c. Demetallation with excess of ethylenediaminetetra-acetic acid (EDTA) provided a simplified BLM analogue, SBLM. A different compound, ABLM, possessing AMPHIS and anthracene as a DNA binding unit was prepared from (**2**) *via* a similar route. The synthetic procedures and spectroscopic data (fast-atom bombardment mass; ^1H and ^{13}C n.m.r.) for SBLM and ABLM will be reported in detail elsewhere.†

The Co^{III} complex was prepared by mixing SBLM or ABLM with a slight excess of $CoCl_2$ in unbuffered aqueous solution adjusted to pH 7.0 with dilute NaOH.[7] Under illumination with 302 nm light from 60 W transilluminator TL-30 for 5 min, supercoiled φX 174 RFI DNA (form I) was almost quantitatively cleaved to nicked circular DNA (form II) in the presence of 5 μM of Co·SBLM (Figure 1, lane 4). A

† *Selected spectral data*: SBLM; 400 MHz ^1H n.m.r. (D_2O) δ 8.63 (s, 1 H), 8.20 (s, 1 H), 8.09 (s, 1 H), 8.06 (t, 1 H, *J* 7.7 Hz), 8.03 (br. d, 1 H, *J* 7.8 Hz), 7.67 (br. d, 1 H, *J* 7.6 Hz), 7.31 (s, 1 H), 4.89 (dd, 1 H, *J* 9.0, 6.7 Hz), 4.61 (d, 1 H, *J* 15 Hz), 4.59 (d, 1 H, *J* 15 Hz), 3.83 (s, 2 H), 3.64 (t, 2 H, *J* 6.6 Hz), 3.63 (t, 2 H, *J* 6.8 Hz), 3.55 (t, 2 H, *J* 6.8 Hz), 3.54 (t, 2 H, *J* 6.6 Hz), 3.43 (dd, 1 H, *J* 15, 6.6 Hz), 3.33 (dd, 1 H, *J* 15, 9.0 Hz), 3.27 (t, 2 H, *J* 6.6 Hz), 3.26 (t, 2 H, *J* 8.3 Hz), 3.18 (t, 2 H, *J* 6.8 Hz), 2.92 (s, 6 H), 2.27 (t, 2 H, *J* 7.3 Hz), 2.10 (m, 2 H), 1.73 (m, 2 H). ABLM; 400 MHz ^1H n.m.r. (D_2O) δ 8.55 (s, 1 H), 8.51 (s, 1 H), 8.07 (s, 1 H), 8.05 (s, 1 H), 7.87—7.98 (m, 4 H), 7.52—7.59 (m, 5 H), 7.15 (s, 1 H), 4.70 (t, 1 H, *J* 7.5 Hz), 4.40 (dd, 2 H, *J* 15, 4.7 Hz), 3.91 (s, 2 H), 3.77—3.85 (m, 4 H), 3.61—3.65 (m, 4 H), 3.13 (dd, 2 H, *J* 24, 7.5 Hz), 3.09 (t, 2 H, *J* 7 Hz), 2.23 (t, 2 H, *J* 5.4 Hz), 1.65 (m, 2 H).

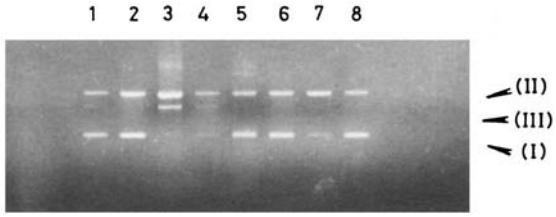


Figure 1. Agarose gel electrophoresis showing results of photochemical cleavage of supercoiled circular φX 174 RFI DNA (form I) into nicked cDNA (form II) and into linear DNA (form III) by photoirradiation in the presence of Co·PEM (lane 3), Co·SBLM (lane 4), Co·ABLM (lane 7), and controls. Reaction mixtures contained 20 μM of DNA in 50 mM sodium cacodylate buffer (pH 7.0). Lane 1, untreated DNA (containing form I and form II DNAs); lane 2, 5 μM $CoCl_2$ + *hν*; lane 3, 2 μM brown Co·PEM + *hν*; lane 4, 5 μM Co·SBLM + *hν*; lane 5, 5 μM Co·SBLM; lane 6, 5 μM Co·ABLM; lane 7, 5 μM Co·ABLM + *hν*; lane 8, 5 μM Co·AMPHIS + *hν*. The reaction mixtures were incubated at 0 °C for 5 min in the dark (lanes 1, 5, and 6) or irradiated at a distance of 6 cm from transilluminator (302 nm) at 0 °C for 5 min (lanes 2, 3, 4, 7, and 8).

similar but less efficient DNA nicking was observed for Co·ABLM at the same concentration (lane 7). Control experiments without light or photoirradiation in the presence of $CoCl_2$ alone did not induce DNA nicking. The efficiency for DNA photocleavage by SBLM is slightly lower than that observed for the brown Co^{III} complex of a natural bleomycin, peplomycin (Co·PEM) (lane 3).[8] The Co complex of the AMPHIS ligand itself did not induce DNA photocleavage at this concentration (lane 8). It is noteworthy that neither Fe·SBLM nor Fe·ABLM showed any DNA cleaving activity in the presence of oxygen and mercaptoethanol.

A preliminary experiment using a 5′-^{32}P end-labelled DNA restriction fragment indicates that photocleavage by Co·SBLM occurred unexpectedly at all bases without any selectivity as judged from the analysis on Maxam–Gilbert sequencing gels, in contrast to the sequence selective cleavage induced by Co·PEM.[8] These results suggest that the bithiazole moiety of bleomycin definitely participates in the sequence-selective cleavage induced by Co·PEM.[8] These results suggest that the bithiazole moiety of bleomycin definitely participates in the sequence-selective binding but does not determine wholly the 5′-G-pyrimidine sites along the DNA strand. A similar non-selective cleavage was also observed for Co·ABLM. Further work including the modification of the ligand for the design of more efficient photochemical DNA cleavers is currently in progress.

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