Photoinduced DNA Strand Scission by Cobalt Bleomycin Green Complex

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Currently, there is considerable interest in molecules that mediate sequence-selective DNA damage upon photoirradiation, as these may provide information useful for the design of novel "photonucleases".[2] The chemically inert bleomycin cobalt complex (Co·BLM) mediates DNA strand scission when photoirradiated at 330–450 nm,[3] whereas irradiation of the Cu or Co complex of a BLM derivative, peplomycin (PEM), at shorter wavelength (~302 nm) resulted in selective transformation of the bithiazole ring.[4]

Two major types of stable Co^{III}·BLM complexes were obtained by mixing BLM with $CoCl_2$ under aerobic conditions.[5] The green and brown complexes were proposed to have hydroperoxide and water as liquids, respectively. Earlier studies indicated the *hν*-dependent, O_2-independent nicking of DNA by the green and brown complexes, as well as the formation of alkali-labile sites and the release of free bases.[3a-c,6]

Reported herein is a more detailed analysis of the chemistry of photoirradiated Co·BLM's. By the use of two BLM congeners and two oligomeric DNA substrates we demonstrate that (i) Fe·BLM and Co·BLM effected oligonucleotide modification at the same sites, (ii) oligonucleotide damage was mediated more efficiently by green Co·BLM than by brown Co·BLM, (iii) oligonucleotide damage by green Co·BLM was O_2 independent, and (iv) the products of Co·BLM-mediated oligonucleotide degradation were limited to free bases and alkali-labile lesions. The accumulated data are consistent with C-4′ hydroxylation of deoxyribose by the green hydroperoxide complex of Co·BLM, with concomitant transformation of the latter to the brown aquo Co·BLM complex.

The green and brown Co^{III}·BLM's were prepared as described[5] and characterized by HPLC, UV, and ^1H NMR. Reaction mixtures containing green Co^{III}·PEM + d(CGCGAATTCGCG) and green Co^{III}·BLM A_2 + d(CGCTTTAAAGCG) were irradiated aerobically at 366 nm. HPLC analysis[8] indicated the formation of cytosine as a major product; neither cytosine propenal nor 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine 3′-(phospho-2″-*O*-glycolate))[7e] was detected in significant amounts, although both were produced in control experiments that employed Fe^{II}·BLM A_2 and Fe^{II}·PEM. Treatment of the reaction mixtures with alkali (0.1 M NaOH, 60 °C, 2 min) effected strand scission at the sites containing alkali-labile lesions; 5′-dGMP was produced from the lesions at cytidine$_{11}$. The quantification of products from one specific experiment that employed green Co^{III}·PEM is given in Table I. As illustrated in the table, degradation of the dodecanucleotide occurred preferentially at C_3 and C_{11} (cf. Scheme I),[8] with lesser amounts of modification at T and A sites.[9] Thus the site selectivity of oligonucleotide modification by Co^{III}·PEM and Co^{III}·BLM A_2 was similar to that observed for the Fe complexes (Table I).[8]

The structure of the alkali-labile product (putative **1**) formed from d(CGCGAATTCGCG) by Co^{III}·PEM was established by treatment of the reaction mixture with alkali (0.1 M NaOH, 60 °C, 2 min) and analysis of the product mixture by HPLC in comparison with authentic dinucleotides **2** (Scheme II).[10] The Co^{III}·PEM-derived sample comigrated with the authentic sample

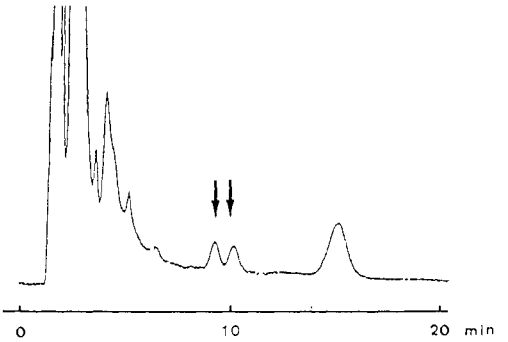
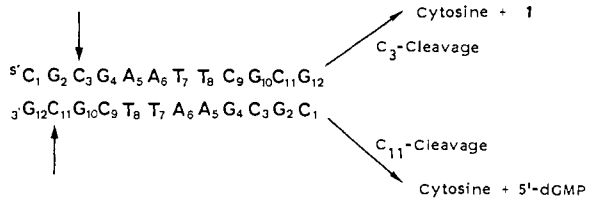


Figure 1. HPLC analysis of products formed by photoreaction of green Co^{III}·PEM with d(CGCGAATTCGCG) followed by alkali treatment. The peaks indicated (arrows) comigrated with authentic diastereomeric dinucleotides **2**. Analysis was carried out on a NOVA $4C_{18}$ column; elution was with 0.1 M ammonium formate containing 2% acetonitrile at a flow rate of 2 mL/min. The same diastereomeric dinucleotides **2** were obtained when d(CGCTTTAAAGCG) was treated analogously with Co^{III}·BLM A_2.

Scheme I. Major Products Formed from d(CGCGAATTCGCG) by Photoirradiated Green Co^{III}·PEM[a]



[a] 5′-dGMP was formed by subsequent alkali treatment. An analogous set of products was formed when d(CGCTTTAAAGCG) was treated with green Co^{III}·BLM A_2.

(1) (a) Kyoto University. (b) University of Virginia. (c) University of California, Davis.

(2) (a) Cadet, J.; Decarroz, C.; Wang, S. Y.; Midda, W. R. *Isr. J. Chem.* **1983**, *23*, 240. (b) Bowler, B. E.; Hallis, L. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 6102. (c) Barton, J. K.; Raphael, A. L. *Ibid.* **1984**, *106*, 2466. (d) Paseuth, D.; Gaudemer, A.; Verhac, J.-B.; Kraljic, I.; Sissoeff, I.; Guille, E. *Photochem. Photobiol.* **1986**, *44*, 717. (e) Mei, H. Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414. (f) Blacker, A. J.; Jazwinski, J.; Lehn, J.-M.; Wilhelm, F. X. *J. Chem. Soc., Chem. Commun.* **1986**, 1035. (g) Blau, W.; Croke, D. T.; Kelly, J. M.; McConnel, D. J.; Ohuigin, C.; van der Putler, W. J. M. *Ibid.* **1987**, 751. (h) Buchardt, O.; Egholm, M.; Karup, G.; Nielsen, P. E. *Ibid.* **1987**, 1696.

(3) (a) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332. (b) Chang, C.-H.; Meares, C. F. *Ibid.* **1984**, *23*, 2268. (c) Wensel, T. G.; Chang, C.-H.; Meares, C. F. *Ibid.* **1985**, *24*, 3060. (d) Subramanian, R.; Meares, C. F. *J. Am. Chem. Soc.* **1986**, *108*, 6427.

(4) (a) Morii, T.; Matsuura, T.; Saito, I.; Suzuki, T.; Kuwahara, J.; Sugiura, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7089. (b) Morii, T.; Saito, I.; Matsuura, T.; Kuwahara, J.; Sugiura, Y. *Ibid.* **1987**, *109*, 938.

(5) Chang, C.-H.; Dallas, J. L.; Meares, C. F. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 959.

(6) Also indicated was the absence of base propenal formation,[7] but the appearance of bands on polyacrylamide gels whose mobility suggested the presence of 3′-phosphoroglycolate termini.[3b]

(7) (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608. (b) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636. (c) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372. (d) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 8612. (e) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735.

(8) (a) Sugiyama, H.; Kilkuski, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765. (b) Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M. *J. Am. Chem. Soc.* **1986**, *108*, 3852.

(9) The specificity for degradation at C_3 and C_{11} was 70% over a range of Co·PEM concentrations. The C_3/C_{11} modification ratio (28:72)[8b] was similar to that observed for Fe^{II}·PEM + d(CGCGAATTCGCG) (17:83); the ratio reported previously[8b] for Fe^{II}·BLM A_2 + d(CGCTTTAAAGCG) was 15:85. No degradation of d(CGCGAATTCGCG) occurred when irradiation was carried out in the absence of Co·PEM.

(10) Sugiyama, H.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58.

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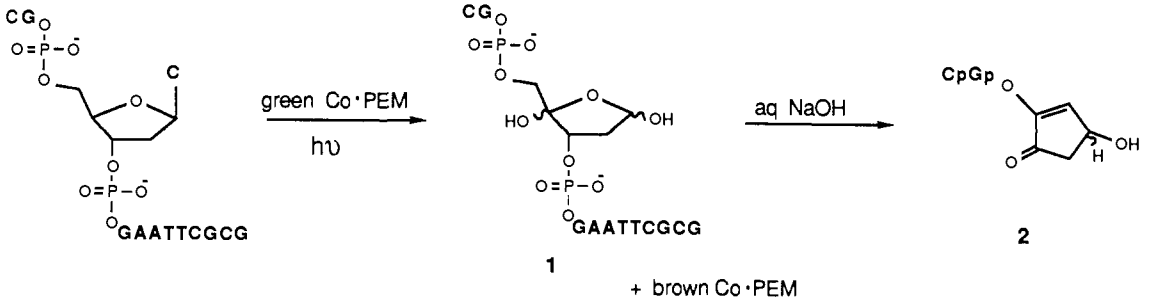
Table I. Quantitative Analysis of Products Formed during the Photoirradiation of Green and Brown Co·PEM Complexes in the Presence of d(CGCGAATTCGCG)[a]

	cytosine, μM	thymine, μM	adenine, μM	dGMP,[b] μM	dodecamer consumed,[c] μM	green complex consumed, μM	brown complex, μM
green Co·PEM	58	15	9	42	67	149	125
brown Co·PEM	8			6	39		248
Fe·PEM[d]	59[e]	12[e]	5[e]				

[a] The 40-μL reaction mixture contained 1 mM d(CGCGAATTCGCG),[c] 50 mM sodium cacodylate (pH 7.0), and 250 μM Co·PEM. After irradiation (Transilluminator TL 33, 5-cm distance) for 1 h at 0 °C (aerobic conditions), analysis was effected by reverse-phase HPLC.[8a] [b] Quantitated as deoxyguanosine after successive treatments of the reaction mixture with base (0.1 M NaOH, 60 °C, 2 min) and alkaline phosphatase. [c] 83 μM dodecanucleotide was used. [d] Control reaction carried out in 60 μL of 50 mM sodium cacodylate (pH 7.0) using 1 mM d(CGCGAATTCGCG) and 300 μM Fe^{II}·PEM. The reaction was carried out for 15 min at 0 °C and then analyzed as above. [e] Base + base propenal.

Scheme II. Plausible Scheme for Formation of Alkali-Labile Lesion **1** and Free Base with Concomitant Conversion of Green Co^{III}·BLM to Brown Co^{III}·BLM



of diastereomers **2** (Figure 1). The absence of cytosine propenal and 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine 3′-(phospho-2″-*O*-glycolate)) suggests that no C-4′ hydroperoxide intermediate was formed;[7a,d,e,8a,10,11] while the chemical transformation(s) leading to the observed products (Scheme I) are not established unequivocally by the present study, C-4′ hydroxylation of the deoxyribose moieties of C_3 and C_{11} would clearly suffice to produce the observed products.

Also studied was the photoirradiation of brown Co^{III}·PEM in the presence of d(CGCGAATTCGCG). This complex was much less efficient than the green complex in mediating degradation of the dodecanucleotide, in spite of the fact that the green and brown complexes of Co^{III}·BLM would be expected to saturate the strong oligonucleotide binding sites under these experimental conditions (Table I).[3c] Degradation of (CGCGAATTCGCG) by green Co^{III}·PEM was accompanied by conversion of the green complex to brown Co^{III}·PEM;[12] analogous degradation by the brown complex did not result in any detectable change in the amount of brown Co^{III}·PEM present at the conclusion of the experiment. Under anaerobic conditions, d(CGCGAATTCGCG) was degraded to the same extent by green Co^{III}·PEM, producing oligonucleotide degradation products and brown Co^{III}·PEM in the same yields observed in the presence of O_2. Further, the quantum yield for the conversion of green Co^{III}·PEM to brown Co^{III}·PEM increased from 1.4×10^{-5} to 6.2×10^{-5} when d(CGCGAATTCGCG) was present.[13] These results suggest strongly that conversion of the green hydroperoxide complex to the brown aquo complex[5] may be associated with ribose C-4′ hydroxylation. Photochemical conversion of green PEM·Co^{III}–OOH to an active oxygen species, perhaps by scission of the O–O bond, could plausibly lead to the observed hydroxylation and production of brown PEM·Co^{III}–OH_2 (Scheme II). Consistent with this suggestion, brown PEM·Co^{III} would be unable to degrade DNA unless it were first converted to green PEM·Co^{III}–OOH, e.g., by photoreduction of Co^{III} to Co^{II} and reoxidation by O_2.[14]

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(11) (a) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694. (b) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104. (c) Saito, I.; Morii, T.; Matsuura, T. *J. Org. Chem.* **1987**, *52*, 1008.

(12) This was verified by HPLC analysis and a 400-MHz ^1H NMR spectrum of the newly formed brown complex following HPLC purification.

(13) The quantum yield measurement was carried out with 366-nm light using phenylglyoxylic acid (ϕ = 0.72 at 365 nm) as an actinometer (Kuhn, H. J.; Defoin, A. *EPA News Letter* **1986**, *26*, 23).

(14) Reduction of brown Co^{III}·PEM with $NaBH_4$ under aerobic conditions led to the formation of green Co^{III}·PEM (20% yield), suggesting that part or all of the oligonucleotide-modifying activity observed with brown Co^{III}·PEM could be due to its conversion to green Co^{III}·PEM under the reaction conditions.

Tautomers of Cytosine by Microwave Spectroscopy

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The relative stability of tautomers of the pyrimidine bases uracil, thymine, and cytosine is of fundamental importance to the structure and functioning of nucleic acids, the occurrence of certain tautomers being suggested as a possible mechanism of spontaneous mutation.[1] Recent developments in the technique of microwave spectroscopy[2] have made it feasible to identify the most stable tautomers of these bases. In our previous studies of uracil[3] and

(1) Pullman, B.; Pullman, A. *Adv. Heterocycl. Chem.* **1971**, *13*, 77-159.
(2) Brown, R. D.; Crofts, J. G.; Godfrey, P. D.; McNaughton, D.; Pierlot, A. P. *J. Mol. Struct.* **1988**, *190*, 185-193.
(3) Brown, R. D.; Godfrey, P. D.; McNaughton, D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1988**, *110*, 2329-2330.

0002-7863/89/1511-2308$01.50/0