Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 378-383

BLEOMYCIN-METAL INTERACTION: FERROUS IRON-INITIATED CHEMILUMINESCENCE

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Received March 15, 1983

Chemiluminescence often accompanies the spontaneous degradation of intermediates in an electronically excited state. The interaction of iron with bleomycin results in the activation of bleomycin to a reactive intermediate which can alter DNA or undergo self-inactivation. This report demonstrates that the interaction of ferrous iron with bleomycin results in chemiluminescence, that this response is iron-specific and that the presence of DNA prevents the generation of chemiluminescence. These observations suggest that the activated bleomycin intermediate may be in an electronically excited state.

The autoxidation of ferrous ions in aqueous solutions gives rise to oxyradicals and in the presence of specific ligands, such as phosphate, chemiluminescence (CL) is observed which is due to the generation of an electronically excited state(s) (1,2). Similarly, the interaction of superoxide or superoxide-derived oxyradicals with oxidizable substrates elicits the formation of hydroperoxide and dioxetane intermediates in an electronically excited state; the spontaneous degradation of these intermediates is accompanied by CL. Of biological relevance is the demonstration that through either an electron transfer reaction or hydrogen abstraction, intermediates in an electronically excited state can alter biomolecules, including nucleic acids (3-8).

Giloni et al. (9) have recently demonstrated that the cleavage of DNA deoxyribose by the antineoplastic antibiotic bleomycin is

0006-291X/83 $1.50

initiated by radical-mediated hydrogen abstraction from the C-4' position of deoxyribose. Bleomycin-mediated DNA deoxyribose cleavage is not due, however, to bleomycin itself but to an "activated" bleomycin intermediate (10). The interaction of iron or iron and hydrogen peroxide with bleomycin elicits the formation of this "activated" bleomycin intermediate which, if formed in the absence of DNA, spontaneously degrades to a product(s) incapable of further altering DNA (10). These molecular actions of bleomycin are analogous in several respects to compounds which are activated to an intermediate in an electronically excited state and since CL is associated with the spontaneous degradation of such intermediates it is theoretically plausible that the "activated" bleomycin intermediate may be in an electronically excited state. Indeed, the results presented in this preliminary communication demonstrate that the interaction of ferrous iron (Fe^{2+}) and bleomycin results in significant CL.

METHODS AND MATERIALS

Chemiluminescence responses were monitored in a Packard liquid scintillation spectrometer (Model 3003) operated at ambient temperature and in the out-of-coincidence mode (11) with the following settings: gain 100%; window A-∞ with discriminators set at 100 to 1000; and input selector 1+2. Reactions (5 ml total volume) were conducted in potassium phosphate buffer (30 mM final concentration) and were initiated by the rapid addition of ferrous sulfate (0.1 mM, dissolved in N_2-purged distilled water) just as the dark-adapted polyethylene vial began to descend into the counting chamber. The counter was then set in the repeat mode at a counting interval of 0.5 min. All additions to the vials as well as the CL counting were conducted in a darkened room. Results are expressed as counts/unit time minus background and data are presented as temporal curves or cumulative CL generated in five consecutive 0.5 min counting intervals. Incorporated into the temporal response curves are the 4 sec delay between the addition of Fe^{2+} or H_2O_2 (5mM) and the positioning of the vial in front of the photomultiplier tubes and the 7 sec delay between counting intervals. Because of these delays, the data are a slight underestimation of the total CL generated.

The bleomycin A_2 isomer (0.025 mM) used in these studies was was dissolved in N_2-purged distilled water and was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan) through the Drug Development Branch of NCI (Bethesda, MD). Calf thymus DNA (0.25 mg/ml) was obtained from Sigma Chemical Co. (St. Louis, MD) and other reagents were of the highest purity commercially available.

RESULTS AND DISCUSSION

Utilizing stop flow spectrometry, an elegant series of studies by Burger et al. (10, 12-14) has advanced the concept that the cleavage of DNA deoxyribose is facilitated by a "activated" bleomycin intermediate which is very labile and self-inactivates. The interaction of Fe^{2+} or Fe^{3+} and H_2O_2 with bleomycin instantaneously intitiates the activation-inactivation of bleomycin or, in the presence of DNA, bleomycin-mediated DNA deoxyribose cleavage. Similarly, the addition of Fe^{2+} to bleomycin A_2 evokes a rapid and distinct CL response that is six times greater than the CL observed in the absence of bleomycin (Figure 1). Fe^{2+}-initiated CL in the absence of bleomycin is consistent with previous reports that addition of Fe^{2+} to aqueous solutions containing phosphate anion results in CL (1,2). In both instances, peak CL is observed within 30 sec following addition of Fe^{2+} and diminishes to background levels by 3 min. Concomitant with the CL response is the autoxidation of Fe^{2+} to Fe^{3+}. Subsequent addition of H_2O_2 elicits a second CL response which also peaks within 30 sec and is 50-fold greater in the presence of bleomycin A_2 (Figure 1). If the interval between

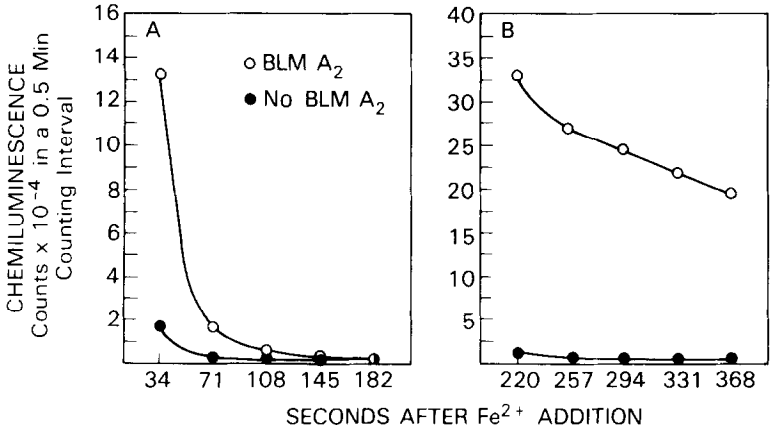

Figure 1. Temporal response curves of CL elicited by the addition of Fe^{2+} (A) and the subsequent addition of H_2O_2 (B). Reactions were conducted in 30 mM potassium phosphate buffer and consisted of 0.1 mM ferrous sulfate, 5 mM H_2O_2 and 0.025 mM BLM A_2.

TABLE 1. METAL SPECIFICITY FOR ELICITING CL FROM THE BLEOMYCIN - METAL INTERACTION

Metal Ion	Reactants			
	Metal Ion Alone[A]	+ H_2O_2[B]	+ $BLMA_2$[A]	+ BLM A_2, H_2O_2[B]
None	-[C]	-	-	0.8
Fe^{2+}	3.0	1.2	15.0	137.5
Cu^{2+}	-	1.5	0.8	-
Co^{2+}	0.8	2.7	-	7.9
Mg^{2+}	-	-	-	-
Mn^{2+}	-	-	-	-
Zn^{2+}	-	-	-	1.3

[A] Cumulative CL (counts x 10^{-4}) generated in five 0.5 min counting intervals elicited by the addition of the indicated metal ion

[B] Cumulative CL (counts x 10^{-4}) generated in five 0.5 min counting intervals elicited by the subsequent addition of H_2O_2

[C] Dashes indicate that no significant CL above background was observed.

the addition of Fe^{2+} and H_2O_2 is decreased than even greater CL is elicited, whereas if this interval is increased less CL results (data not shown).

Although bleomycin binds cobalt, copper, iron, magnesium or zinc cations, it is the iron-bleomycin interaction which initiates the activation of the bleomycin molecule to a DNA damaging intermediate (10). The data presented in Table 1 clearly demonstrates that CL arising from a bleomycin-metal interaction is likewise greatest with Fe^{2+}. Upon subsequent addition of H_2O_2, cobalt and zinc yield CL in the presence of bleomycin; however, this response is miniscule relative to the iron-bleomycin-H_2O_2 interaction.

Faljoni et al. (6) have shown that DNA considerably diminishes the CL resulting from electronically excited acetone and that alterations in DNA coincides with generation of this intermediate. Likewise, DNA obviates the CL arising from bleomycin whether it is initiated by Fe^{2+} or H_2O_2 (Figure 2). Analysis of these reaction mixtures for thiobarbituric acid reactive products (TBAR) that originate from deoxyribose cleavage (9) indicate that in the presence

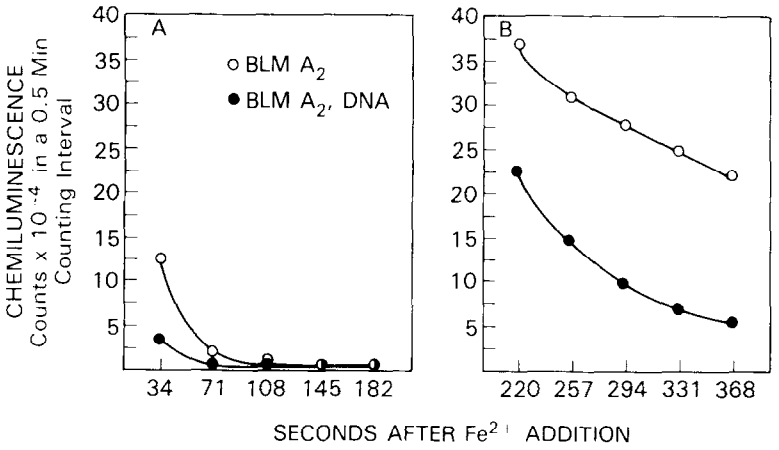

Figure 2. Effect of calf thymus DNA (0.25 mg/ml) on the CL elicited by the addition of Fe^{2+} (A) and the subsequent addition of H_2O_2 (B). Concentrations of reactants were the same as in Figure 1.

of bleomycin addition of Fe^{2+} yields TBAR and that the subsequent addition of H_2O_2, as illustrated in figures 1 and 2, elicits a further increase in bleomycin-dependent TBAR (data not shown). Thus, the reduction of CL by DNA, in the presence of bleomycin and Fe^{2+}, coincides with increased TBAR and demonstrates that the

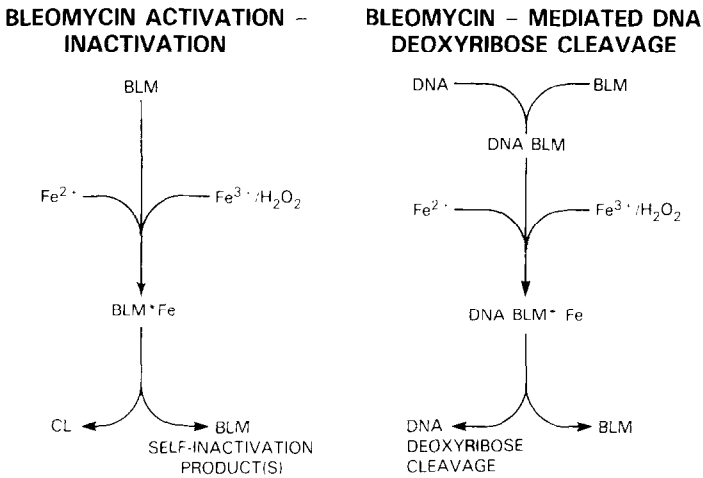

Figure 3. Proposed hypothesis as to the relationship between the generation of the activated bleomycin intermediate, chemiluminescence (CL), and bleomycin-mediated deoxyribose cleavage. The interaction of Fe^{2+} or Fe^{3+}/H_2O_2 with BLM elicits the generation of an activated bleomycin intermediate in an electronically excited state (BLM*). In the absence of suitable target molecules, BLM* undergoes self-inactivation by a process which is accompanied by CL. On the other hand, the interaction of BLM* with the deoxyribose of DNA allows BLM* to return to ground state thereby preventing the self-inactivation of the bleomycin molecule and allowing BLM to undergo subsequent activation.

diminution of CL is not due to the failure of DNA-bound bleomycin to react with Fe^{2+}. These observations suggest then that there may be a common intermediate to both the DNA deoxyribose cleavage reaction and the CL response. In this regard, the results of the spectral studies by Burger et al. (10,12-14) demonstrate quite clearly that DNA does not prevent the iron-initiated activation of bleomycin but does prevent the self-inactivation reaction.

Thus, it was in the context of these spectral studies on the activation-inactivation of bleomycin (10,12-14), the demonstration that electronically excited intermediates can alter biomolecules (3-8), and the observations presented in this communication that we proposed that the bleomycin molecule may be activated to an intermediate in an electronically excited state and that this electronically excited bleomycin intermediate is capable of directly damaging DNA (15). Figure 3 illustrates this proposal.

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