

DIRECT COMPARISON OF OXYGEN TRANSFER
BY IRON BLEOMYCIN AND ZINC BLEOMYCIN

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Abstract: A direct comparison has been made of the oxygen transfer abilities of Fe(III), Zn(II), and their respective bleomycin (BLM) complexes. These (chelated) metal ions were treated with iodosobenzene in aqueous methanol containing *cis* or *trans*-stilbene. It was found that oxygen transfer was observed only when redox active Fe(III)-BLM was employed. Benzaldehyde was the only product observed in the above systems in the absence of Fe(III)-BLM; the available evidence suggested that it resulted from electron abstraction by iodosobenzene alone. The results of the present study suggest that a metal-oxo intermediate is the species most likely to be involved in oxygen transfer following admixture of Fe(III)-BLM and iodosobenzene in the presence of *cis*-stilbene.

Bleomycin-mediated DNA strand scission¹ via a process that also requires O₂ and a redox active metal ion such as Fe(II),² Cu(I),³ and Mn(II)⁴ is well established. The Fe(III),⁵ Cu(II),^{3b} and Mn(III)^{4a} complexes of BLM also mediated DNA strand scission in the presence of oxygen surrogates such as iodosobenzene. In addition to effecting DNA strand scission, metallobleomycins activated with C₆H₅IO and other oxygen surrogates have been shown to mediate the oxidative transformation of a number of olefinic substrates.^{3c,4a,5,6} Certain of these transformations, e.g., the epoxidation of *cis*-stilbene, closely resemble substrate oxidations by cytochrome P-450⁷ and model systems incorporating metalloporphyrins.⁸ Although not established definitively, the available evidence is consistent with the formation of a high valent metal-oxo intermediate. Metal-oxo intermediates have also been implicated in the catalytic transfer of oxygen from simple metal salts to substrates⁹.

In a recent report, Moriarty et al.¹⁰ suggested that metal ions, either alone or as bleomycin complexes, act simply to coordinate iodosobenzene in an aqueous methanolic medium. The coordinated complex formed was suggested to create an electrophilic intermediate which could combine with olefinic substrates. Data was presented which indicated that Zn(II)-BLM catalyzed the epoxidation of *cis* and *trans*-stilbene; both were reported to yield predominantly *trans*-stilbene oxide.¹⁰ The use of Fe(ClO₄)₃·9H₂O alone, in amounts approximately 10⁴ times greater than those employed as metallobleomycins, also gave low yields of *trans*-stilbene oxide starting from either *cis* or *trans*-stilbene. Fe(III) was also reported to catalyze the epoxidation of styrene and chalcone. Because the conclusions of this report¹⁰ imply that a high valent metal-oxo species is not a likely intermediate in the transfer of oxygen to substrate in these systems⁶, and bear relevance to work ongoing in other laboratories,¹¹ we have reinvestigated certain of the findings of the report.

The data presented in Table I indicate that under our experimental conditions a 1:1 complex of redox-active¹² Fe(III)-bleomycin was the only tested species capable of producing epoxides and deoxybenzoin from *cis*-stilbene. Further, the 10:1 ratio of *cis*:*trans* epoxides formed demonstrated that this reaction was stereospecific. In the presence of *trans*-stilbene, a much poorer substrate for Fe(III)-BLM, trace amounts of epoxide were still

observed. However, in the presence of redox inactive Zn(II)-BLM, or Zn(II) or Fe(III) ions alone, no epoxides or deoxybenzoin were produced from either cis or trans-stilbene.

Table I. Oxidation of cis or trans-Stilbene in the Presence of Fe(III).BLM + C₆H₅IO or Zn(II).BLM + C₆H₅IO^a

Metal/ Metallo BLM	Substrate	Products (μmol) ^b				Total Yield (%) ^c
		<u>trans</u> - Stilbene oxide	<u>cis</u> - Stilbene oxide	Benzal- dehyde	Deoxy- benzoin	
<u>cis</u> -Stilbene						
Fe(III).BLM		0.31	7.64	2.40	1.16	415
Zn(II).BLM		—	—	0.27	—	10
Fe(III) ^{d,e}		—	—	0.17	—	6
— ^e		—	—	0.30	—	11
<u>trans</u> -Stilbene						
Fe(III).BLM		— ^f	— ^f	1.86	—	67
Zn(II).BLM		—	—	1.16	—	42
—		—	—	0.81	—	30

^aReactions employed 2.76 μmol of BLM in 1.0 mL of 6:4 CH₃OH-H₂O, which was treated with Fe(III)(ClO₄)₃·9H₂O, Zn(II)SO₄·7H₂O or Zn(II)(ClO₄)₂·6H₂O in 0.5 mL of CH₃OH, and then with 40 mg (0.22 mmol) of cis- or trans-stilbene in 2.0 mL of CH₃OH. Prior to initiation of individual reactions, each reaction mixture was rendered anaerobic by alternate vacuum/argon purges in a Thunberg tube; the reactions were carried out under argon. Iodosobenzene (15 mg; 68 μmol) was then added dropwise as a 1.0-mL CH₃OH solution over 10-15 min. After 2 hr at 25°C, the reaction mixtures were diluted with H₂O and extracted with CH₂Cl₂. Product analysis was conducted by HPLC using an Alltech Applied Sciences analytical silica gel (5μ) column. Products were eluted with 25% chloroform in cyclohexane and detected at 254 nm. Retention times (min): trans-stilbene oxide, 10.4; cis-stilbene oxide, 15.4; benzaldehyde, 25.8; deoxybenzoin, 48. trans-Stilbene formation was monitored using gas chromatography with an Alltech OV-17 column equipped with a thermal conductivity detector employing decanol as an internal standard. Retention times (min): Decanol; 2.3; cis-stilbene, 7.5; trans-stilbene, 8.7. ^bAs little as 0.1 μmol of cis-stilbene oxide and 0.01 μmol of trans-stilbene oxide could have been detected. ^cPercent yield based on 2.76 μmol metalloBLM. ^dReaction employed 13.8 μmol of Fe(III). ^eYield based on assumed 2.76 μmol of limiting reagent to facilitate comparison. ^fTrace amount of product observed; too little to quantitate accurately.

Benzaldehyde alone was produced in low yields in the absence of Fe(III).BLM and the amount of benzaldehyde produced in such cases was apparently dependent only on the presence of C₆H₅IO and dioxygen, (see Tables I and II). The amount of benzaldehyde produced from trans-stilbene was greater than the amount

produced from cis isomer; however, C₆H₅CHO production still depended only on C₆H₅IO, an observation that probably reflects differences in reactivity between cis and trans-stilbene.

The mechanism of C₆H₅CHO production from cis-stilbene by Fe(III).BLM has recently been studied.^{6b} The data were consistent with a scheme involving initial electron abstraction from cis-stilbene to form the cation radical, the latter of which could capture dioxygen under aerobic conditions, ultimately yielding benzaldehyde as the major product. The present results suggest that the same mechanism can operate, albeit with lesser efficiency, in the absence of BLM or added metal ions, and that this BLM-independent transformation required C₆H₅IO and was facilitated by added O₂. Experimental evidence consistent with the intermediacy of a stilbene cation radical in the BLM-independent process included (i) the formation of trans-stilbene as the major product when cis-stilbene and C₆H₅IO were admixed under scrupulously anaerobic conditions, (ii) an increase in C₆H₅CHO production under aerobic conditions (Tables I and II), and (iii) the incorporation of ¹⁸O into C₆H₅CHO when ¹⁸O₂ was employed¹³.

In no case was Zn(II) + C₆H₅IO or Zn(II).BLM + C₆H₅IO found to result in the formation of any product from cis or trans-stilbene to a greater extent than C₆H₅IO alone. Moreover, unlike the products of oxygen transfer obtained from stilbene with Fe(III).BLM + oxidant, in which the oxygen atoms in the product were derived from the oxidant,¹⁴ the only product formed in the presence of Zn(II) + C₆H₅IO or Zn(II).BLM + C₆H₅IO derived its oxygen atom from O₂.

The present study reaffirms the previous conclusion that oxygen transfer in such biomimetic systems is not solely a function of the Lewis acidity of the metal ions involved.^{9b} Further, the strong dependence of oxygen transfer on the presence of a redox-active metal ion is consistent with the intermediacy of high valent metal-oxo species in metallobleomycin-mediated oxygenation reactions.

Table II. Production Benzaldehyde from cis-Stilbene by C₆H₅IO + O₂ in the Presence of Metallobleomycins^a

Metal/Metallobleomycin	Benzaldehyde (μmol)
Fe(III).BLM ^b	4.50
Zn(II).BLM	0.60
Zn(II)	0.82
Zn(II) ^c	0.76
BLM	0.86
—	0.45

^aAll reactions contained 2.76 μmol of BLM or metal ion, except as noted. ^bAlso produced were cis and trans-stilbene oxides and deoxybenzoin in % yields comparable to those in Table I. ^cReaction employed 11.04 μmol of Zn(II).

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