Chemical Reactivities of Bleomycin*

(Received for publication, March 11, 1985)
Guy Padbury and Stephen S. Sligar†
From the Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

Ferric bleomycin was tested for its ability to catalyze a set of six oxidative reactions characteristic of the heme-containing proteins, cytochrome P-450 and chloroperoxidase. These reactions included peroxo-acid decarboxylation and aliphatic hydroxylation as typical cytochrome P-450 chemistries. Peroxycid-supported oxygen evolution and hydrogen peroxide-mediated chlorination were utilized as characteristic chloroperoxidase reactivities. A typical peroxidative reaction and heteroatom dealkylation, common to both O₂ activating enzymes, were also studied.

Bleomycin was found to catalyze peroxidation of o-dianisidine. The ferric drug complex was found competent in carrying out N-demethylation of N,N-di-methylamino when peroxides or peroxycids or iodosobenzene were used as the oxidants. N-Demethylation was not achieved when N,N-dimethylamino-N-oxide was substituted as the oxidant under similar conditions. Hydroxylation of cumene and decarboxylation of phenylperacetic acid were not found to be catalyzed by bleomycin. Oxygen evolution from m-chloroperoxybenzoic acid and chlorination of monochlorodimedone from chloride ion and hydrogen peroxide were found to be catalyzed by bleomycin. Cytochrome P-450<sub>cam</sub> was also evaluated for O₂ evolution, and halogenation activity and was found not to demonstrate such reactivities.

The results of this initial survey, along with those of previous studies, appear to indicate that the chemical reactivity of bleomycin can be more closely aligned with the reactivities demonstrated by chloroperoxidase than those of cytochrome P-450.

The bleomycins constitute a family of glycopeptide antibiotics which differ only in their terminal amide functional groups (1) and are employed clinically for the treatment of certain carcinomas and lymphomas (2). The therapeutic efficacy of the bleomycins is believed to be related to their ability to degrade DNA in vivo (3), a process that has been shown, in vitro, to proceed in the presence of appropriate metal ions and a source of oxygen (4–8).

These antineoplastic agents are isolated as one-to-one cop-

* This study was supported by National Institutes of Health Grant GM31756 to S. G. S. Some portions of this work have been presented at the 56th Southeastern Regional Meeting of the American Chemical Society, October 24–26, 1984, Raleigh, NC and the 58th Annual Meeting of The American Societies for Experimental Biology Meeting, April 21–26, 1985, Anaheim, CA. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Recipient of Career Development Award AM 01160.

† Recipient of Career Development Award AM 01160.

per complexes from cultures of Streptomyces verticillus (9, 10) but are found to readily form complexes with a variety of metals (11). Extensive investigations on these metallo-drug complexes, particularly the iron complexes, have led to the observation of several parallels between the bleomycins and the cytochromes P-450, a family of heme-containing monooxygenases (12).

Although structurally dissimilar, with bleomycin lacking a conjugated polyaromatic structure like heme and thiolate ligation to the metal (11, 13), iron drug complexes, reminiscent of the cytochromes P-450, are readily formed which can bind dioxygen and carry out site-specific oxidations (5, 14). Both systems activate dioxygen by parallel pathways (12, 15) and combine with O₂ to form an oxygenated complex (12, 16). Similarities between the two systems in terms of the electronic parameters, crystal field analysis of certain intermediates of the activation cycle, and interactions with various oxygen analogues have been established using a variety of spectral methods including Mössbauer (17), ¹H NMR (18), EPR (15, 19–21), and optical spectroscopies (20, 22). Further interrelations have been observed relating to aerobic activation with NADPH cytochrome P-450 reductase (23–25), anaerobic activation by peroxides and other oxygen surrogates (5, 15), the effects of certain inhibitors (20, 22), and the reactivity towards certain olefinic substrates (5, 7). Bleomycin is not unique in its complementarity with cytochrome P-450. Chloroperoxidase, a heme protein isolated from the mold Caldariomycetes fumago that catalyzes biological halogenation reactions, also demonstrates remarkable correlations with cytochrome P-450 in terms of its physiochemical properties as evaluated by optical absorption (26–29), ESR (29), Mössbauer (30, 31), resonance Raman (32, 33), and magnetic circular dichroism spectroscopies (34). Therefore, indirectly, bleomycin can also be likened to chloroperoxidase.

Based on the strong physical evidence which has indicated the similarities between bleomycin and the cytochromes P-450, and circuitously chloroperoxidase, we have sought to carry out a preliminary survey of the ability of ferric bleomycin to catalyze some of the reactions catalyzed by the two heme proteins. The results of this initial evaluation show that the chemical reactivities demonstrated by bleomycin appear to align more closely with those demonstrated by chloroperoxidase than those of cytochrome P-450.

MATERIALS AND METHODS

Bleomycin sulfate (Blenoxane<sup>®</sup>) was a gift of Bristol Laboratories and was used without further purification. A molecular weight of 1550 was assumed for the drug and ε<sub>280</sub> = 1.45 × 10<sup>4</sup> M⁻¹ cm⁻¹. Ferric bleomycin was prepared as described by Burger et al. (20).

Chloride peroxidase (EC 1.11.1.10), a gift of Dr. L. P. Hager's laboratory, was purified to an RZ of greater than 1.2 by the method of Gunsalus and Wagner (36). Oxidized cytochrome P-450<sub>cam</sub> without substrate (P-450<sub>cam</sub>)

† The abbreviations used are: P-450<sub>cam</sub>, oxidized camphor-hydroxy-
were performed on a Varian Model 3700 gas chromatograph using commercially from Sigma, Aldrich, Fisher, or

detect any benzyl alcohol, the decarboxylation product of the peroxyacid. In experiments incubating ferric bleomycin with

decondition, GC analysis of the reaction mixtures were unable to detect any benzyl alcohol, the decarboxylation product of the

peroxyacid. In experiments incubating ferric bleomycin with the hydroxylatable substrate cumene and suitable oxidant

under various reaction conditions, the hydroxylated products, 2-phenyl-2-propanol or 2-phenyl-1-propanol, was not detected by GC evaluation.

Among the unique reactions catalyzed by chloroperoxidase is the evolution of oxygen from substituted peroxides and peroxycacid (47). Oxygen evolution from meta-chloroperoxybenzoic acid was found to be readily catalyzed by bleomycin. Results shown in Fig. 1 compare the moles of oxygen evolved as a function of the moles of bleomycin or chloroperoxidase present in the reaction mixture at a constant m-CPBA concentration. The leveling off of the oxygen evolution at the higher levels of bleomycin is attributed to insufficient excess of the peroxycacid to maintain the pseudo-first order reaction conditions. Chloroperoxidase is also found to show the same leveling effect at much higher enzyme concentrations (data not shown). Fig. 2 shows the oxygen evolved as a function of the m-chloroperoxybenzoic acid concentration at a fixed bleomycin level. This relationship was found to be linear over the concentrations of peroxycacid studied. The lower limit was determined by the sensitivity of the O2 electrode used in the assay, while the upper limit was a function of the solubility of the m-CPBA. Oxygen evolution could not be detected when the m-CPBA was replaced by peracetic acid, phenylperacetic acid, ethyl hydroperoxide, or t-butyl hydroperoxide in the bleomycin system. Cytochrome P-450 sign was not found to catalyze O2 evolution from the various alkyl peroxides and peroxycacids at any detectable levels in our hands, which may be a result of this enzyme's rapid autoxidation rate.

Chloroperoxidase also has the capability of catalyzing the

![Graph showing oxygen evolution as a function of chloroperoxidase concentration](image-url)
confirmed as dichlorodimedone, with a trace amount of di-
medone noted by GC analysis against synthesized standards.
No reactivity was found when cytochrome P-450 \( \text{P-450}_{\text{OH}} \) was evalu-
ated for its ability to catalyze the same reaction under condi-
tions similar to those of the chloroperoxidase assay. The ob-
served initial rates reported for the different systems in
Table I cannot be compared quantitatively; however, empiri-
cally they appear to indicate that bleomycin is much less
efficient than chloroperoxidase in catalyzing this reaction.

Bleomycin was found to catalyze N-demethylation of \( N,N \)-
dimethylaniline, a reaction that has previously been shown to
be efficiently mediated by cytochrome P-450 (48) and chloro-
peroxidase (49). Several oxidants were found to be able to
support the N-demethylation of \( N,N \)-dimethylaniline with
iodosobenzene and with ascorbate and \( \text{O}_{2} \). N-Demethy-
lization was not achieved in systems where \( N,N \)-dimethylaniline-\( N \)-oxide was substituted as the oxidant. This observation parallels the results of Hol-
genberg and co-workers (49) on chloroperoxidase. Cytochromes
P-450 \( \text{P-450}_{\text{OH}} \) and P-450 \( \text{P-450}_{\text{LM}} \) have been reported to efficiently utilize
the exogenous oxidant DMANO in the N-demethylation of
DMA (51).

Both heme proteins and bleomycin were found to carry out
the peroxidation of \( o \)-dianisidine. Based on observed initial
rates, chloroperoxidase was found to catalyze this reaction most efficiently \((1.83 \times 10^{-6}) \) nmol of \( \text{H}_{2}\text{O}_{2} \) consumed/min
/nmol of chloroperoxidase) followed by bleomycin \((3.39 \times 10^{-4}) \)
nmol of \( \text{H}_{2}\text{O}_{2} \) consumed/min/nmol of bleomycin) and cyto-
chrome P-450 \( \text{P-450}_{\text{OH}} \) \((1.57 \times 10^{-4}) \) nmol of \( \text{H}_{2}\text{O}_{2} \) consumed/min/nmol of cytochrome P-450), respectively. In each of the above
cases, the reaction was found to be dependent on \( \text{H}_{2}\text{O}_{2} \) and
the respective catalyst. Again, empirically, chloroperoxidase
is much more effective in catalyzing this reaction than bleo-
mycin. The low activity of the cytochrome P-450 \( \text{P-450}_{\text{OH}} \) can be
attributed to the high substrate specificity and rapid autoxi-
dation rate of the enzyme.

Table III summarizes the results of this initial survey. The
table shows that, for the cross-section of oxidative reactivities
studied, bleomycin demonstrates the same reactivity as
chloroperoxidase, including the two rather unique chloro-
peroxidase reactivities of halogenation and peroxacyl (m-
CPBA)-mediated oxygen evolution.

The unique new reactivities of bleomycin shown in this
initial evaluation definitely indicate that further and more
extensive studies are merited.

Acknowledgments—We wish to express our appreciation to Dr.
William T. Bradner and Linda C. Sanders of Bristol-Myers Company

![Graph](image-url)
for their generous gift of the bleomix, and to Karen Cummings and Steve Blanke of Dr. L. P. Hager’s laboratory for supplying the chloroperoxidase and dichlorodimedone and for their insightful discussions. Our appreciation is also extended to Dr. Ralph Murray for his invaluable assistance with the synthesis procedures and to William Atkins for his help with the cytochrome P-450 experiments.

REFERENCES

1. Fuji, A., Takita, T., Maeda, K., and Umezawa, H. (1973) J. Antibiot. (Tokyo) 26, 396-399
2. Crooke, S. T., and Bradner, W. T. (1977) J. Med. 7, 333-342
3. Suzuki, H., Nagai, K., Yamaki, H., Tanaka, N., and Umezawa, H. (1986) J. Antibiot. (Tokyo) 21, 379-386
4. Sausville, E. A., Peisach, J., and Horwitz, S. (1976) Biochem. Biophys. Res. Commun. 76, 814-822
5. Murugesan, N., Ehrenfeld, G., and Hecht, S. M. (1982) J. Biol. Chem. 257, 8600-8603
6. Chang, C.-H., and Meares, C. (1982) Biochemistry 21, 6322-6334
7. Ehrenfeld, G. M., Murugesan, N., and Hecht, S. M. (1984) Inorg. Chem. 23, 1486-1498
8. Burger, R. M., Freedman, J. H., Horwitz, S. B., and Peisach, J. (1984) Inorg. Chem. 23, 2215-2217
9. Umezawa, H., Maeda, K., Takeuchi, T., and Okami, Y. (1966) J. Antibiot. (Tokyo) 19, 200-209
10. Umezawa, H., Suhara, Y., Takita, T., and Maeda, K. (1966) J. Antibiot. (Tokyo) 19, 210-215
11. Dabrowiak, J. C. (1980) J. Inorg. Biochem. 13, 317-337
12. White, R. E., and Coo, M. J. (1980) Annu. Rev. Biochem. 49, 315-336
13. Takita, T. (1978) in Bleomycin: Chemical, Biochemical and Biological Aspects (Hecht, S. M., ed) pp. 37-47, Springer Verlag, Berlin
14. Giloni, L., Takahashi, M., Johnson, F., Iden, C., and Grollman, A. P. (1981) J. Biol. Chem. 256, 8608-8615
15. Burger, R. M., Peisach, J., and Horwitz, S. B. (1981) J. Biol. Chem. 256, 1168-1164
16. Burger, R. M., Horwitz, S. B., Peisach, J., and Wittenberg, J. B. (1979) J. Biol. Chem. 254, 12299-12302
17. Burger, R. M., Kent, T. A., Horwitz, S. B., Munck, E., and Peisach, J. (1983) J. Biol. Chem. 258, 1559-1564
18. Sugiyama, Y., Suzuki, T., Murakawa, Y., Umezawa, Y., Takita, T., and Umezawa, H. (1981) J. Antibiot. (Tokyo) 34, 1232-1236
19. Sugiyama, Y., and Kikuchi, T. (1978) J. Antibiot. (Tokyo) 31, 1310-1312
20. Burger, R. M., Peisach, J., Blumberg, W. E., and Horwitz, S. B. (1979) J. Biol. Chem. 254, 10906-10912
21. Antholine, W. E., and Petering, D. H. (1979) Biochim. Biophys. Acta 50, 384-389
22. Sugiyama, Y., Suzuki, T., Kawabe, H., Tanaka, H., and Watanabe, K. (1982) Biochem. Biophys. Acta 716, 38-44
23. Scheule, M. E., Kappus, H., Thyszen, D., and Schmidt, C. G. (1982) Biochem. Pharmacol. 30, 3385-3388
24. Scheule, M. E., and Kappus, H. (1983) in Oxidation Radicals in Chemistry and Biology, Proceedings of the 3rd International Conference (Bors, W., Saran, M., and Tait, D., eds) pp. 425-435, deGruyter, Berlin
25. Kikukawa, R. E., MacDonald, T. L., and Hecht, S. M. (1984) Biochemistry 23, 6165-6171
26. Hollenberg, P. F., and Hager, L. P. (1973) J. Biol. Chem. 248, 2630-2633
27. Palec, M. R., Rutter, R., Araiso, T., Hager, L. P., and Dunford, H. B. (1980) Biochem. Biophys. Res. Commun. 94, 1123-1127
28. Sone, M., Dawson, J. J., and Hager, L. P. (1984) J. Biol. Chem. 259, 13209-13216
29. Hollenberg, P. F., Hager, L. P., Blumberg, W. E., and Peisach, J. (1980) J. Biol. Chem. 255, 4801-4807
30. Champion, P. M., Munck, E., Drubrunner, P. G., Hollenberg, P. F., and Hager, L. P. (1979) Biochemistry 12, 436-435
31. Champion, P. M., Chang, C.-H., and Peisach, J. (1970) J. Biol. Chem. 254, 587-590
32. Miwa, G. T., Walsh, J. S., Kedderis, G. L., and Hollenberg, P. F. (1978) Biochemistry 17, 4694-4692
33. Rembe, R. D., Champion, P. M., Fitcher, D. B., and Hager, L. P. (1979) Biochemistry 18, 2280-2290
34. Dawson, J. H., Trumpel, J. R., Barth, G., Linder, R. E., Bunnenberg, E., Djerassi, C., Chang, R., and Hager, L. P. (1976) J. Am. Chem. Soc. 98, 3709-3710
35. Shahangian, S., and Hager, L. P. (1981) J. Biol. Chem. 256, 6034-6040
36. Gunsalus, I. C., and Wagner, G. C. (1978) Methods Enzymol. 52, 168-188
37. McDonald, R. N., Steppe, R. N., and Dorsey, J. E. (1970) Org. Synth. 50, 15-18
38. White, R. E., Sligar, S. G., and Coon, M. J. (1980) J. Biol. Chem. 255, 11109-11111
39. Martin, A. S. (1960) in Organic Analysis (Mitchell, J., Jr., Koh, M. I., Proskauer, E. S., and Weissberger, A., eds) Vol. 4, pp. 15-16, Interscience, New York
40. Cotton, M. L., and Dunford, H. B. (1973) Can. J. Chem. 51, 582-587
41. Manthey, J. A., and Hager, L. P. (1981) J. Biol. Chem. 256, 11232-11238
42. Hager, L. P., Morris, D. R., Brown, F. S., and Eberwein, H. (1966) J. Biol. Chem. 241, 1769-1777
43. Seltzmann, H., and Sharek, J. G. (1973) Org. Syn. Collect. 5, 658-659
44. Craig, J. C., and Murpohan, K. C. (1970) J. Org. Chem. 35, 1712-1722
45. Worthington Enzyme Manual (1972) pp. 43-44, Worthington Biochemical Corporation, Freehold, NJ
46. McCarthy, M. B., and White, R. E. (1983) J. Biol. Chem. 258, 9153-9158
47. Hager, L. P., Doubek, D. L., Silverstein, R. M., Lee, T. T., Thomas, J. A., Hargis, J. H., and Martin, J. C. (1973) in Oxidation and Related Redox Systems (King, T. E., Mason, H. S., and Morrison, M., eds) University Park Press, Baltimore
48. Miwa, G. T., Walsh, J. S., Kedderis, G. L., and Hollenberg, P. T. (1983) J. Biol. Chem. 258, 14445-14449
49. Kedderis, G. L., Koop, D. R., and Hager, L. P. (1980) J. Biol. Chem. 255, 10174-10182
50. Murugesan, N., and Hecht, S. M. (1985) J. Am. Chem. Soc. 107, 493-500
51. Heinmember, D. C., Murray, R. I., Egeberg, K. D., and Sligar, S. G. (1984) J. Am. Chem. Soc. 106, 1514-1516