mum $\chi_m|J|/Ng^2\mu_B^2$ and temperature $k_BT_m/|J|S(S+1)$ for different values and signs of J and J'. Then the susceptibility was developed as a function of temperature near the maximum for suitable values of the interchain interaction.

From the experimental measurements we get from the average susceptibility in the three main directions

$$\frac{\chi_m|J|}{Ng^2\mu_B^2} = 0.105 \pm 0.001 \qquad \frac{k_BT_m}{|J|S(S+1)} = 1.00 \pm 0.05$$

The fact that the reduced susceptibility is higher than the corresponding one for the pure linear-chain antiferromagnet implies the presence of some ferromagnetic interaction. (Recall that the intrachain interaction was found from the high-temperature analysis to be antiferromagnetic.)

Taking $J < 0$ and $J' > 0$, we obtained the same value of R from both experimental parameters χ_m and T_m. The rounded maximum typical of an antiferromagnet does not define accurately the temperature of the maximum and then allows a range of values for R, and we get $R = -0.05 \pm 0.05$. The value of the susceptibility at the maximum is known with more precision, and it yields $R = -0.06 \pm 0.02$. The interchain interaction is small enough for the compound to behave as a good linear chain above the temperature $2T_m$ yet is sufficiently high to allow the observation of the crossover effects in the paramagnetic region and make quantitative comparisons with the theory. The detailed analysis will be presented elsewhere, together with complementary measurements of the heat capacity.

Acknowledgment. The work in Chicago has been supported by the Solid State Chemistry Program, Division of Materials Research, National Science Foundation, under Grants DMR-7906119 and DMR-8211237. The work in Zaragoza has been supported by Comisiòn Asesora de Investigaciòn Cientifica y Tècnica.

Registry No. $K_2[FeF_5(H_2O)]$, 35848-60-3.

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Received February 14, 1984

DNA Degradation by Manganese(II)–Bleomycin plus Peroxide

Sir:

The DNA-cleaving activity of bleomycin,[1] an antitumor glycopeptide antibiotic, had been shown in vitro to depend on the formation of activated bleomycin, a drug complex containing Fe(III) and oxygen.[2,3] This complex can be formed with Fe(III) and peroxide, Fe(III) plus reductants and O_2, or Fe(II) and O_2. Other metals gave no detectable DNA degradation in O_2-dependent reactions, and many inhibit the iron-requiring reaction.[4] We now report DNA degradation by Mn(II)–bleomycin in the presence of H_2O_2. This activity is not due to endogenous iron salts and differs from that of iron–bleomycin in several respects. DNA does not inhibit the Mn(II)–bleomycin reaction, as it does the iron–bleomycin reaction, but optimal activity is only 1–3% of that of the Fe(III)-drug complex with H_2O_2. DNA products include free bases and base propenals,[5] as with iron bleomycin, but in different proportions. Aerobic solutions of reducing agents such as 2-mercaptoethanol cannot substitute for peroxide in the reaction with Mn(II) as they do in the reaction with Fe(III). In this respect, Mn(II)–bleomycin parallels the behavior of Mn(II)–cytochrome P-450, which cannot be activated reductively but can be activated with peroxides.[6]

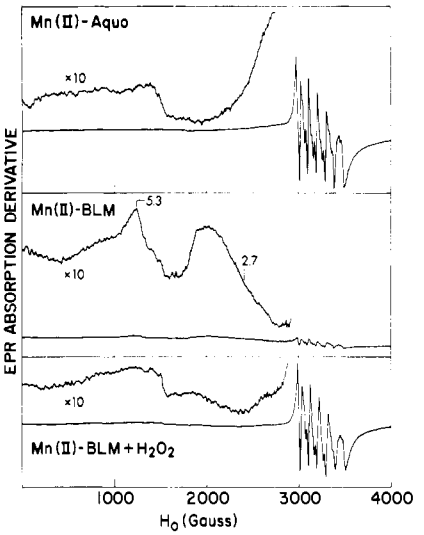

Figure 1. EPR spectrum of Mn(II)–bleomycin. Samples contain 0.2 mM Mn(II), 10 mM Hepes buffer (pH 8.2), 50% (v/v) ethylene glycol and, where indicated, 0.3 mM bleomycin or bleomycin plus 100 mM H_2O_2. Spectra were taken at 77 K on a Varian Model E-12 EPR spectrometer operated at 9.07 GHz, 10-mW power, with 20-G field modulation. Identical spectrometer gain settings were used for all samples; ×10 denotes spectra taken with 10-fold enhanced gain. Values of g are given in the middle panel. An additional broad, $g_{min} = 1.3$ feature seen with Mn(II)–bleomycin is not shown here.

Like many transition-metal ions, Mn(II) forms a complex with bleomycin, as indicated by its EPR spectrum (Figure 1). When bleomycin is added to Mn(II) solutions at pH >7.5, the characteristic resonance of aquo–Mn(II) near $g = 2$ gives way to one with features near $g = 5.3$, 2.7, and 1.3. Subsequent addition of H_2O_2 changes the EPR spectrum to resemble that of Mn(II)–aquo near $g = 2$ but with broad differences apparent elsewhere at higher spectrometer gain. This material differs from Mn(II)–aquo and Mn(II)–bleomycin in producing base propenal from DNA.

When Mn(II) was surveyed for activity with bleomycin, a spectrophotometric assay we had used for iron–bleomycin activity[7] was adapted. This assay is based on the reaction of

(1) Umezawa, H. In "Anticancer Agents Based on Natural Product Models"; Cassady, J. M., Duros, J. D., Eds.; Academic Press: New York, 1980; pp 147–166. Suzuki, H.; Nagai, K.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot.* **1969**, *22*, 446–448.
(2) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814–822. Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 576–582.
(3) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636–11644.
(4) Dabrowiak, J. C. *Adv. Inorg. Chem.* **1982**, *4*, 69–113.
(5) The trivial name base propenal denotes compounds of the form 3-(pyridin-1-yl)-2-propenal and 3-(purin-9-yl)-2-propenal, characterized as bleomycin-induced DNA products by: Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615.
(6) Gelb, M. H.; Toscano, W. A., Jr.; Sligar, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5758–5762.
(7) Burger, R. M.; Peisach, J.; Blumberg, W. E.; Horwitz, S. B. *J. Biol. Chem.* **1979**, *254*, 10906–10912.

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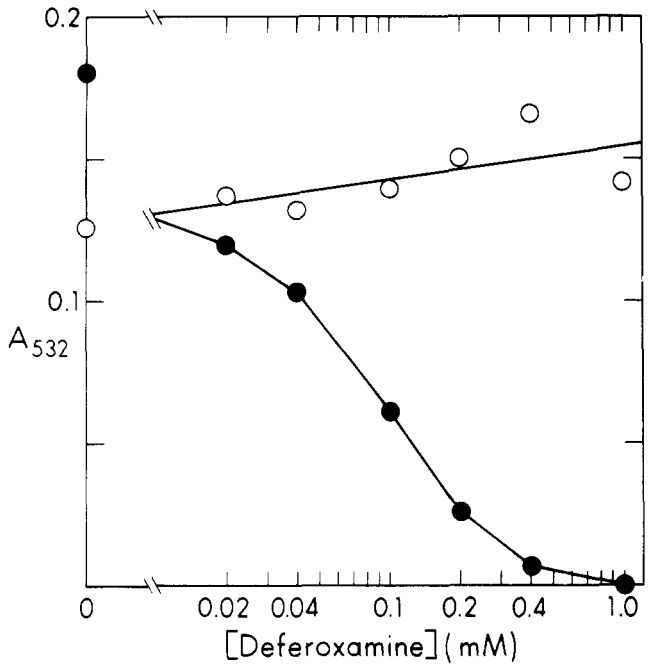

Figure 2. DNA-degrading activity of Mn(II)–bleomycin is refractory to the specific Fe(III) chelator deferoxamine. Reaction mixtures consist of 0.1 mL of 0.03 mM Fe(III)–bleomycin and 0.1 mM DNA (●) or 0.4 mL of 0.5 mM Mn(II)–bleomycin and 6 mM DNA (○), both buffered at pH 8.2 with 15 mM Hepes. Reactions were initiated by the final addition of 25 mM H_2O_2. Deferoxamine, where indicated, was added 1 h prior to the H_2O_2. Both preincubation and incubation were conducted at 20 °C. Reactions were terminated at 2 min by addition of the 2-thiobarbituric acid reagent. Reaction velocities for Fe(III)–bleomycin and Mn(II)–bleomycin are respectively 160 and 1.7 mmol/min/mol of metal in the absence of deferoxamine. Mn(II)–bleomycin solutions were prepared from Fisher Certified Mn(II)–acetate, which contains 5 ppm Fe.

base propenals[5,8] with 2-thiobarbituric acid to form a pink adduct identical with that formed with malondialdehyde ($\epsilon = 1.6 \times 10^5$ M^{-1} cm^{-1}, $\lambda_{max} = 532$ nm).[9] We obtained this same chromophore from mixtures of DNA reacted with Mn(II)–bleomycin and H_2O_2. The specific rate and yield of chromogen formation with Mn(II)–bleomycin did not exceed about 1% of those with Fe(III)–bleomycin, and therefore larger, more concentrated reaction mixtures were used. Thus, in a typical experiment (Figure 2), 0.4-mL reaction mixtures containing 6 mM DNA, 0.5 mM Mn(II), and 0.6 mM bleomycin yield about as much of the 532-nm chromophore as 0.1 mL of 0.1 mM DNA, 0.03 mM Fe(III), and 0.036 mM bleomycin, upon incubation at pH 8.2 (15 mM Hepes[10]) with 25 mM H_2O_2. In both mixtures, reactions were terminated by dilution to 0.8 mL with a solution of 41 mM 2-thiobarbituric acid, 5 mM EDTA, and 10 mM HCl and were then heated to 92 °C for 10 min. The omission of bleomycin, H_2O_2, or DNA from the Mn(II) reaction mixture eliminated all chromogen formation, and omission of Mn(II) eliminated >90%. Some marginal activity is expected in mixtures receiving no metal, due to endogenous iron,[11] but this activity, unlike that of Mn(II)–bleomycin, is also obtained with reductants substituted for H_2O_2. Another difference between activity with Mn(II) and with Fe(III) is seen at pH 7: DNA in excess of 3 times the concentration of Fe(III)–bleomycin is strongly inhibitory[3] but the activity of Mn(II)–bleomycin increases with the DNA:drug ratio up to at least 20.

The relatively slight activity with Mn(II) made it important to demonstrate by other means that this activity did not derive from endogenous iron. For this purpose the strong, specific Fe(III) chelator deferoxamine[12] was used to demonstrate that iron complexation had no effect on mixtures containing Mn(II)–bleomycin. In the experiment of Figure 2, reaction mixtures of comparable activity, containing either Fe(III)– or Mn(II)–bleomycin, were titrated with deferoxamine, added 1 h prior to the peroxide-initiated reaction, in order to permit the slow complexation of Fe(III) by the chelator to occur.[13] The reaction with Mn(II)–bleomycin is unaffected or slightly enhanced by deferoxamine at a concentration sufficient to eliminate all detectable activity in the Fe(III)–bleomycin reaction. This level of deferoxamine (1 mM) exceeds by 33-fold the concentration of Fe(III), which gives activity comparable to that with the Mn(II) used in this experiment, and exceeds by 20-fold the half-inhibitory concentration of deferoxamine in the Fe(III) reaction.

The detailed mechanism of Mn(II)–bleomycin activation is not as well understood as that of iron–bleomycin. Under the conditions described for Figure 2, the reaction stops after 3 min. Addition of 20 mM 2-mercaptoethanol to a reaction in progress stops it within 15 s. Although specific activities above 4 mmol/mol/min have been obtained, saturating concentrations of DNA and H_2O_2 appear to exceed the capacity of the assay system. It is therefore difficult to assess the rate-limiting processes or identify activation intermediates.

The reaction products are incompletely characterized, but they include free bases and base propenals, as revealed by reversed-phase thin-layer chromatography.[14] [^{3}H]thymine-labeled DNA[14] was incubated with Mn(II)–bleomycin and H_2O_2, and authentic [^{14}C]thymine was added as an internal standard. The DNA was removed by ethanol precipitation just before sample application. The ratio of free base to base propenal, about 5, is significantly higher than with aerobic iron–bleomycin reactions, which produce approximately equal yields of each.[14,15] The precision of this determination with Mn(II)–bleomycin is limited by the 1000-fold excess of unreacted DNA, which is accompanied by a small fraction of radioactive free base and uncharacterized DNA breakdown products.

Many transition metals bind to bleomycin and several then react with oxygen species.[4] Of these, Fe(II) and Fe(III) confer the greatest DNA-degrading activity. Co(II)–bleomycin forms a transient 1:1 and a stable 2:1 complex with O_2.[16] In our hands, Co(II)–bleomycin is inactive in DNA cleavage. Aerobic Co(III)–bleomycin has shown some DNA-cleaving activity when exposed to light,[17] but neither the mechanism nor the role of O_2 has been determined. Cu(I)–bleomycin is oxidized by O_2 but does not cleave DNA unless it has been activated with iodosobenzene.[18] Fe(III)–bleomycin plus iodosobenzene does not cleave DNA but can epoxidize *cis*-stilbene.[18]

Mn(II) most nearly resembles Fe(III) in reacting with bleomycin and H_2O_2 to produce free bases and base propenals

(8) These are stoichiometric products of bleomycin-induced DNA strand scission: Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 8612–8614.
(9) Waravdekar, V. S.; Saslaw, L. D. *J. Biol. Chem.* **1959**, *234*, 1945–1950.
(10) Hepes is Ultrol brand 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid from Calbiochem-Behring.
(11) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740–2746.
(12) Emery, T. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1971**, *35*, 135–183. Given to us by CIBA as "Desferal Mesylate".
(13) Arif Kazmi, S.; McArdle, J. V. *J. Inorg. Biochem.* **1981**, *15*, 153–162.
(14) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832–11838.
(15) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372–3375.
(16) Sugiura, Y. *J. Antibiot.* **1978**, *31*, 1206–1208. Albertini, J. P.; Garnier-Suillerot, A. *Biochemistry* **1982**, *21*, 6777–6782.
(17) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332–6334.
(18) Murugesan, N.; Ehrenfeld, G. M.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 8600–8603.

from DNA, but there are differences in the reaction properties and product ratios. The similar reactivity of peroxide with Mn(II)- and Fe(III)-bleomycin is also seen with the mechanistically similar enzyme cytochrome P-450,[6] which has been prepared with Mn(II) substituted for Fe(III). The Mn(II)-substituted protein is active, but with altered substrate specificity and kinetic properties. Mn(II)-substituted cytochrome P-450, while active with peroxide, is no longer active with O_2, just like Mn(II)-bleomycin. Both the coordination of iron ligands[7,19] and activation pathways for cytochrome P-450[20] and bleomycin[3] are quite similar, notwithstanding bleomycin's lack of either a thiol or an aromatic macrocyclic moiety, like a porphyrin.[1] Activated bleomycin appears to initially execute a 4'-deoxyribose hydrogen abstraction,[21] such as cytochrome P-450 does with other substrates.[20] Since there is spectral evidence that a monooxygenated species of Mn(II)-substituted cytochrome P-450 may be relatively long-lived,[6] we are hopeful that an analogous oxygen complex, if produced, may be evident with Mn(II)-bleomycin as well. The form of oxygen ligated by Mn(II)-bleomycin, however, need not be the same as that bound to Fe(III)-bleomycin.

Acknowledgment. This is Communication No. 273 from the Joan and Lester Avnet Institute of Molecular Biology. This work was supported by NIH Grant CA 15714 and American Cancer Society Grant CH-86 (S.B.H.) and NIH Grant HL-13399 (J.P.). R.M.B. is a Leukemia Society of America Scholar.

Registry No. H_2O_2, 7722-84-1.

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Received March 6, 1984

(19) Burger, R. M.; Kent, T. A.; Horwitz, S. B.; Münck, E.; Peisach, J. *J. Biol. Chem.* **1983**, *258*, 1559-1564.
(20) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315-356.
(21) Wu, J. C.; Kozarich, J. W.; Stubbe, J. A. *J. Biol. Chem.* **1983**, *258*, 4694-4697.

Articles

Contribution from the Institut für Anorganische und Analytische Chemie, Freie Universität Berlin, 1000 Berlin 33, West Germany, and Department of Chemistry, Clemson University, Clemson, South Carolina 29631

Pentafluoroselenium Isocyanate and Pentafluorotellurium Isocyanate, $F_5Se-N=C=O$ and $F_5Te-N=C=O$

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Received October 21, 1983

The previously unknown $F_5Se-N=C=O$ is prepared from $Xe(OSeF_5)_2$ and HCN; $F_5Te-N=C=O$ can be prepared similarly but also by more straightforward routes.[2] Compared with that of $F_5S-N=C=O$, the isocyanate reactivity is reduced in $F_5Te-N=C=O$ and even more so in $F_5Se-N=C=O$.

Introduction

$F_5S-N=C=O$ has been known for many years.[3,4] It exhibits a chemistry of a typical isocyanate, so it adds to protic substrates to form urethanes or to carbonyls to form azomethines.[5] Quite in contrast to this, the tellurium-nitrogen chemistry of this type was rather underdeveloped and the selenium-nitrogen chemistry was nonexistent. $TeF_5-N=C=O$ has recently been prepared from $F_5Te-NH-Si(CH_3)_3$;[2] the latter, from TeF_6.[6] Since SeF_6 does not undergo any controlled substitution reactions, no direct key for the preparation of $F_5SeN<$ systems was available. We thus prepared $F_5Se-N=C=O$ from $Xe(OSeF_5)_5$ and HCN. This reaction was suggested by an earlier reaction of $Xe(OTeF_5)_2$ with HCN, which resulted in TeF_5NCO, in an attempt to prepare a xenon-carbon bond. As discussion will show, it was not at all clear from the beginning that we obtained an isocyanate rather than a cyanate. This problem has now been solved by an electron-diffraction study of $F_5S-N=C=O$, $F_5Se-N=C=O$, and $F_5Te-N=C=O$, which proved all materials to be isocyanates.[7]

Experimental Section

General Data. ^1H and ^{19}F NMR spectra were recorded on a Varian EM 360 instrument. IR spectra were taken on a Beckman IR 12; Raman spectra, on a Cary 82 with Ar-laser excitation. Mass spectra were recorded on a Varian MAT CH 5 or Varian MAT 711. Moisture-sensitive materials were handled in a Braun glovebox with a water level of about 1 ppm.

Reagents. $Xe(OTeF_5)_2$, $Hg(OSeF_5)_2$, $Hg(OTeF_5)_2$,[8] and XeF_2[10] were prepared by literature methods. $SeOF_2$ was obtained from SeO_2 and SF_4.[9]

Xenon Bis(pentafluoroselenate), $Xe(OSeF_5)_2$.[8] In the glovebox, a 100-mL stainless-steel vessel was filled with 30 g of XeF_2. An open Teflon-FEP tube containing 16 g of $SeOF_2$ was then placed carefully upright in the stainless steel vessel on top of the XeF_2. The vessel was closed and cooled to -30 °C for 1 h. Then the vessel was shaken for 2 days at room temperature. The gas contents of the vessel were mainly xenon, which is blown out, purified by washing and drying, and used again for the preparation of XeF_2.

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(2) Hartl, H.; Huppman, P.; Lentz, D.; Seppelt, K. *Inorg. Chem.* **1983**, *22*, 2183.
(3) Tullock, W. C.; Coffman, D. D.; Muetterties, E. L. *J. Am. Chem. Soc.* **1964**, *86*, 357.
(4) Duncan, L. C.; Rhyne, T. C.; Clifford, A. F.; Shaddix, R. E.; Thompson, J. W. *Inorg. Nucl. Chem. Lett.* **1967**, *3*, 133.
(5) (a) Thrasher, J.; Howell, J. L; Clifford, A. F. *J. Fluorine Chem.* **1984**, *24*, 431. (b) Thrasher, J. S.; Howell, J. L.; Clifford, A. F. *Inorg. Chem.* **1982**, *21*, 1616. (c) Thrasher, J. S. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, 1981. (d) Howell, J. L. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, 1978.
(6) Seppelt, K. *Inorg. Chem.* **1973**, *12*, 2837.
(7) Oberhammer, H.; Seppelt, K.; Mews, R. *J. Mol. Struct.* **1983**, *101*, 325.
(8) Seppelt, K.; Nothe, D. *Inorg. Chem.* **1973**, *12*, 2727.
(9) Lentz, D.; Seppelt, K. *Inorg. Synth.*, in press.
(10) Malm, J. G.; Selig, H.; Jortner, J.; Rice, S. A. *Chem. Rev.* **1965**, *65*, 199.

0020-1669/84/1323-2217$01.50/0