unlike the unstabilized C's, discriminate very much more and inversely, which is strange,[21] but accords with **1** (also a stabilized metal carbene) reacting much more quickly with acetylenes than with alkenes.[1,7,22,23]

Acknowledgment. We are grateful to Yuan-Chi Ying for assisting with the experiments with cyclooctene and to the National Science Foundation (CHE-77-22726) and the U.S. Office of Naval Research for support.

(21) It correlates with nucleophilic reactivity: Dickstein, J. I.; Miller, S. I. In "Chemistry of the Carbon-Carbon Triple Bond"; Patai, S., Ed.; Wiley: New York, 1978; Vol. 2, p 813 ff.
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(23) P's are presumably less stabilized than **1**, which is why they initiate metatheses that **1** does not.

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Unusual Interaction of Iron-Bleomycin with Cyanide

Sir:

The bleomycin (BLM)-Fe(II) complex appears to act by reducing molecular oxygen to a more reactive species in the vicinity of a susceptible DNA bond, resulting in its cleavage.[1] Optical, ^{1}H NMR, and ESR spectroscopies have demonstrated that the BLM-Fe(II) complex forms a complex with either CO, isocyanide, or NO.[2] The activity of BLM, monitored by the production of malondialdehyde, is reversibly inhibited by CO or ethyl isocyanide.[2a] These substances are oxygen antagonists, just as with heme oxygenases. It is known that CN ion binds more strongly to ferric than to ferrous hemes[3] and inhibits the reaction by heme oxygenases.[4] In contrast, cyanide ion enhances the BLM activity against DNA cleavage.[2a]

On the basis of several spectroscopic results, Scheme I has been proposed for the BLM-iron interaction.[5] The reaction of the high-spin BLM-Fe(II) complex with O_2 yields the corresponding Fe(III) complex, which again undergoes reduction by a reducing agent. Recent stopped-flow optical spectroscopy indicated the presence of a short-lived intermediate, an oxygenated BLM-Fe(II) complex.[6] A stable, diamagnetic BLM-Fe(II)-CO adduct complex has been shown by a proton NMR study.[2b]

In connection with the cyanide enhancement of BLM activity, we have investigated the interaction of the BLM-iron complexes with CN ion by optical, ^{1}H NMR, and ESR spectroscopies and found an unusual cyanide interaction of iron-BLM.

The Fe(II) and Fe(III) complexes of bleomycin-A_2 (BLM) were prepared according to the previous procedure.[5] 220-MHz FT ^{1}H NMR (sample concentration 10 mM) and X-band ESR (1 mM) spectra were recorded with Varian HR-220 and JES-FE-3X spectrometers, respectively.

The addition of NaCN to the BLM-Fe(II) and BLM-Fe(III) complexes induced color changes from pink to blue-violet and from yellow-brown to red-brown, respectively. Table I summarizes the visible spectral characteristics of these iron complexes. The results of optical titration at pH 6.9 indicated that the BLM-Fe(II) (or -Fe(III)) complex forms a 1:1 cyanide adduct complex.

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(2) (a) Burger, R. M.; Peisach, J.; Blumberg, W. E.; Horwitz, S. B. *J. Biol. Chem.* **1979**, *254*, 10906-12. (b) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616-20. (c) Sugiura, Y.; Ishizu, K. *J. Inorg. Biochem.* **1979**, *11*, 171-80.
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(4) Ohnishi, T.; Hirata, F.; Hayaishi, O. *J. Biol. Chem.* **1977**, *252*, 4643-7.
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(6) Burger, R. M.; Horwitz, S. B.; Peisach, J.; Wittenberg, J. B. *J. Biol. Chem.* **1979**, *254*, 12299-302.

Scheme I

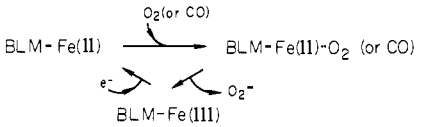

Table I. Effect of Cyanide Ion on Visible Absorption Spectra of Bleomycin-Iron Complexes

complex	λ_{max}, nm (ϵ)
BLM-Fe(II)	476 (380)
BLM-Fe(II) + CN^-	385 (1600), 570 (1400), 605 (1300)
BLM-Fe(III)	365 (2000), 384 (1900)
BLM-Fe(III) + CN^-	390 (2000), 465 (800)

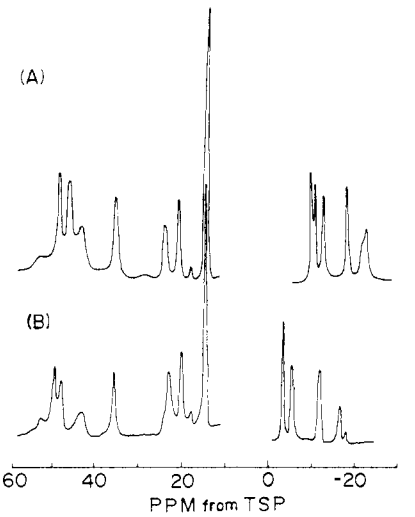

Figure 1. Proton NMR spectra of the BLM-Fe(II) complex (A) and the BLM-Fe(II)-CN complex (B) at pD 7.3 and 25 °C.

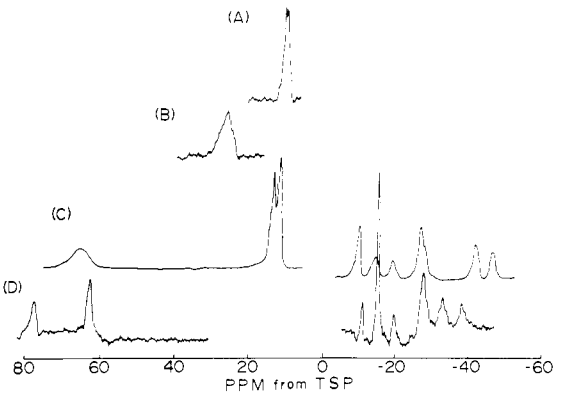

Figure 2. Proton NMR spectra of the BLM-Fe(III) complex (A), the BLM-Fe(III)-CH_3NH_2 complex (B), the BLM-Fe(III)-CN complex (C), and the BLM-Fe(III)-CH_3NH_2-CN complex (D) at pD 7.3 and 25 °C.

Figure 1 shows the 220-MHz FT ^{1}H NMR spectrum of the 1:1:1 BLM-Fe(II)-CN complex together with that of the original BLM-Fe(II) complex. Although the spectra show similar patterns, the magnitude of the proton paramagnetic shifts of the BLM-Fe(II)-CN complex differs somewhat from that of the original BLM-Fe(II) complex and the former signals appear at lower field. In contrast with the absence of proton paramagnetic signals for the corresponding CO (or C_2H_5NC) adduct ($S = 0$), the large paramagnetic shifts of the BLM-Fe(II)-CN adduct suggest the presence of a high-spin ferrous iron ($S = 2$).[7]

Figure 2 illustrates the 220-MHz FT ^{1}H NMR spectra of the

(7) The Mössbauer parameters of the BLM-Fe(II)-CN adduct are also typical of high-spin Fe(II) and are somewhat different from those of the original BLM-Fe(II) complex. On the other hand, the BLM-Fe(III)-CN adduct has Mössbauer constants characteristic of high-spin Fe(III) (unpublished results).

0002-7863/80/1502-7944$01.00/0

BLM–Fe(III) complexes and their 1:1 cyanide adducts at 25 °C. The small proton paramagnetic shifts of the BLM–Fe(III) complex (11.6 ppm) and the BLM–Fe(III)–CN_3NH_2 complex (25.1 ppm) indicate low-spin ferric ($S = {}^1/_2$) state. Indeed, the ESR features of these Fe(III) complexes at 77 K are characteristic of a rhombic low-spin type: $g_x = 1.893$, $g_y = 2.185$, and $g_z = 2.431$ for the BLM–Fe(III) complex and $g_x = 1.847$, $g_y = 2.179$, $g_z = 2.540$ for its methylamine adduct.[5] On the other hand, the addition of cyanide ion to the BLM–Fe(III) and BLM–Fe(III)–CH_3NH_2 complexes produced a drastic change in the original ^1H NMR spectra, and new proton peaks appeared in the lower and higher field regions. The magnitude of the chemical shifts (over ±50 ppm) strongly suggests a high-spin ferric type for these cyano complexes.[8] CO, NO, and C_2H_5NC bind to the BLM–Fe(II) complex as a sixth ligand to form low-spin ferrous adduct complexes,[2] and several nitrogenous bases also coordinate to the BLM–Fe(III) complex to give low-spin ferric adducts.[5] If CN ion similarly binds to the vacant sixth coordination site of the BLM–iron complex, which has a rigid square-pyramidal arrangement with the 5–5–5–6 ring member,[5] the cyanide adducts would be expected to have a low-spin iron state.

We have found that the BLM–Fe(II)–O_2 system efficiently produces oxygen radicals such as $O_2^{-}\cdot$ and ·OH.[9] Indeed, similar ESR spin-trapping experiments using *N-tert*-butyl α-phenyl nitrone have shown that the addition of CO (or C_2H_5NC) strongly interferes with O_2 activation by the BLM–Fe(II) complex, but stoichiometric CN addition slightly increases the production of oxygen radicals in comparison with the original BLM–Fe(II) system.

BLM–iron complexes and hemoproteins apparently display similarities in the binding of oxygen antagonists (CO, NO, and C_2H_5NC) and in external nitrogenous bases, but the interaction of CN ion is remarkably different. In general, cyanide interferes with reaction of heme oxygenases, and CN adducts of ferric hemoproteins are of low-spin type. The present unusual behavior of CN ion toward the BLM–iron complexes appears to be responsible for the cyanide enhancement of the BLM activity against DNA. A detailed investigation of the CN interaction and the complete assignment of the proton signals are now under way.

Acknowledgment. Gratitude is due to Professor Hamao Umezawa for encouragement, Dr. Tomohisa Takita for pertinent advice, and M. Ohara for comments on the manuscript. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

(8) When CN ion was added to BLM–Fe(III) and its CH_3NH_2 adduct complexes, the typical low-spin ESR signals disappeared and a new broad ESR absorption near $g = 4$ appeared. However, quantitative consideration of this signal is difficult at present because of its complexity.

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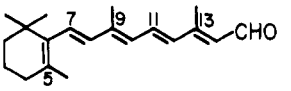
An External Point-Charge Model for Bacteriorhodopsin To Account for Its Purple Color

Sir:

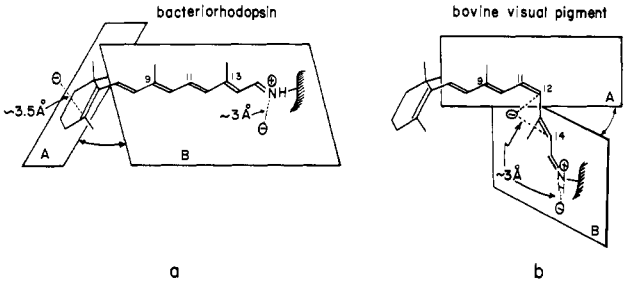
Bacteriorhodopsin is the pigment contained in the purple membrane of *Halobacterium halobium*, a halophilic bacterium, and it functions as a light-driven proton pump.[1] The chromophore is a retinal bound to the ε-amino group of lysine via a protonated Schiff base linkage (SBH^+).[2] *all-trans*-Retinal is the chromophore of the light-adapted form of bacteriorhodopsin, λ_{max} 570 nm, while a 1:1 mixture of *all-trans*- and 13-*cis*-retinals is the chromophore of the dark-adapted form, λ_{max} 560 nm.[3] Since the maximum of the SBH^+, with *n*-$BuNH_2$, in MeOH[4,5] is at 440 nm,

Table I. Absorption Maxima (nm) of Chromophores and Pigments[a]



chromophore	all-trans	5,6-2H	7,8-2H	9,10-2H	11,12-2H
aldehyde[b]	381	370	338	278	236
SBH^+ [c]	440	425[d]	385	322	270
calcd	*460*	*441*	*398*	*342*	
bacteriorhodopsins	560	476	400	325	*f*
calcd	*576*[e]	*485*[e]	*415*[e]	*347*[e]	
opsin shift (cm^{-1})[g]	4870	2500	1000	300	
calcd	*4400*	*2100*	*1000*	*420*	
bovine rhodopsins[h]	485[d]	460[d]	420[d]	345	315
opsin shift (cm^{-1})[g]	2100	1800	1700	2100	5300

[a] The trans isomers were incubated with bacterioopsin in the dark, 1 h, room temperature, 67 mM pH 7.0 phosphate buffer. [b] In MeOH. The maxima of the aldehyde-containing all-trans chromophores are listed. [c] Protonated Schiff base with *n*-$BuNH_2$, in MeOH; see ref 5. [d] Values are for the 9-cis isomers. Values for the rhodopsins are for the 9-cis isomers because the 9-*cis*-retinals are more readily synthesized then the 11-cis isomers. For 9,10- and 11,12-dihydro chromophores the values are for the "all-trans" isomers; here flexibility of the single bond allows the all-trans isomer to form pigments (see ref 6). [e] Calculated shifts for model shown in Figure 1a. See ref 8 for details of calculation. [f] Could not be measured due to overlap with protein absorption. [g] See text. [h] Data from ref 6; measured in 0.5% digitonin, phosphate buffer, pH 7.0.



a b

Figure 1. Models for the wavelength-determining electrostatic interactions in the chromophore binding site of (a) bacteriorhodopsin and (b) bovine rhodopsin. The ring-chain angle was *arbitrarily* set at 45° for both rhodopsin and bacteriorhodopsin; this is the value derived from NMR solution studies[10] and theoretical calculations.[10] The existence of a counterion near the protonated nitrogen is assumed. A second negative charge is located in the case of bacteriorhodopsin (Figure 1a) ~3.5 Å above C-5. The position of the charge depicted in Figure 1a, however, is only one example of a location consistent with the experimental results; other locations of the negative charge near the β-ionone ring are also possible. For bovine rhodopsin (Figure 1b) the negative charge is placed ~3 Å from C-12 and C-14.

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