containing **1**, should contain 5′-GXA-3′, and the cross-linking ODN should contain 3′-CY**1**-5′, where X and Y are complementary nucleotides.[17]

Several observations can be made regarding the significance of these results. First, the cleavage at 51 or 58 °C after 48 h is virtually quantitiative while absolute specificity is retained, making this method appropriate for the sequence-specific cleavage of single-stranded DNA. Second, the demonstrated site of alkylation suggests that the original Brookes and Lawley hypothesis regarding cross-linking across GC doublets may require revision; perhaps the actual cross-link is from G to G in GXC triplets. Third, the ability of the modified ODN to selectively alkylate the desired target without random alkylation on noncomplementary nucleic acids supports their use in chemotherapeutic agents if the cross-linking rate can be improved.

(17) To examine the regiospecificity of the reaction, we prepared a 24-mer target containing the sequence 5′-CGA-3′ and a cross-linking 14-mer ODN containing 3′-GC**1**-5′. No cross-linked or cleaved target strand was found when these were incubated under conditions similar to those in Figure 1, emphasizing the specificity of the reaction for the *second* G from **1**.

Detection of a New Signal in the EPR Spectrum of Vanadium Nitrogenase from *Azotobacter vinelandii*

Brian J. Hales,*,† Anne E. True,‡ and Brian M. Hoffman*,‡

Department of Chemistry
Louisiana State University
Baton Rouge, Louisiana 70803
Department of Chemistry
Northwestern University
Evanston, Illinois 60201
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It is now well established that *Azotobacter vinelandii*[1,2] and *Azotobacter chroococcum*[3,4] can express alternative nitrogen-fixing enzymes that contain vanadium instead of molybdenum. The paramagnetism of the vanadium-containing protein of this enzyme from *A. vinelandii*[5] (called Av1′)[6] previously has been investigated and shown to exhibit at least two distinct EPR signals. In this communication, we report the discovery of a third EPR signal in the spectrum of the vanadium enzyme, which is difficult to detect in the absorption mode but is easily observed in the dispersion mode at pumped helium temperatures.

The first-derivative absorption EPR spectrum of Av1′ recorded at 14 K and 10 mW clearly shows two distinctly different signals, Figure 1, part A. The first signal (S1) has axial symmetry at $g \sim 2$ and most likely is associated with the $S = {}^1/_2$ spin system of a reduced Fe–S cluster. The second signal (S2), centered at $g = 5.5$, represents the low-field inflections of the ground-state and first-excited-state transitions of the two Kramers doublets of an $S = {}^3/_2$ spin system and has been tentatively assigned to a protein-bound VFe cofactor, analogous to the spin system of the MoFe cofactor of the conventional nitrogenase enzyme. It has been noted[5] that as the sample temperature is lowered and the incident microwave power increased (Figure 1, parts B and C), the recorded spectrum changes in several ways. Firstly, S2 changes in shape with temperature because it is composed of inflections from both the ground state and the first excited state,

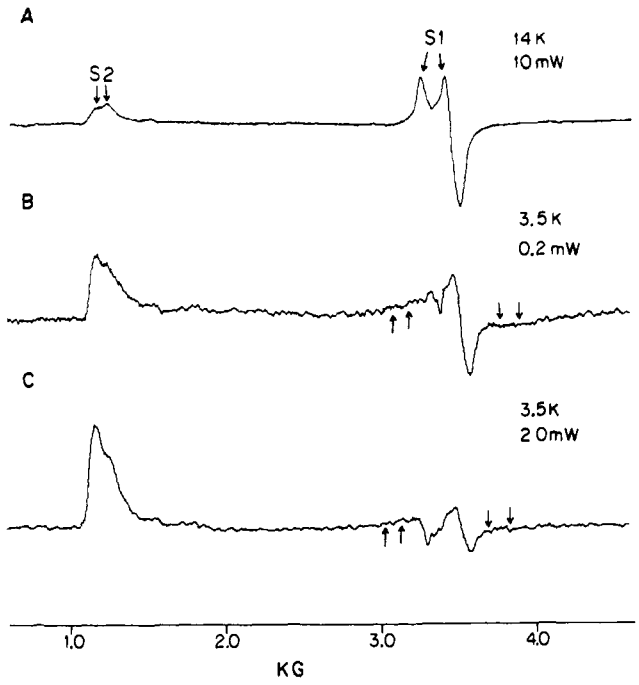

Figure 1. First-derivative absorption spectra of nitrogenase VFe protein (25 mM Tris buffer, pH 7.4, containing 0.25 M NaCl and 2 mM dithionite) from *Azotobacter vinelandii* showing the effects of varying temperature and incident microwave power on signals S1 and S2. Conditions: protein concentration, 25 mg mL^{-1}; sp act., 200 nmol of C_2H_2 reduced min^{-1} (mg of protein)$^{-1}$; modulation amplitude, 20 G; 100-kHz field modulation; frequency, 9.32 GHz; temperature and microwave power as indicated. Arrows in B and C point to the weak change in the baseline due to the center inflection of S3. This inflection occurs about $g \sim 2$ and, because it is the only portion of the S3 absorption with a major derivative amplitude change, is observable in this presentation under the conditions of high power and low temperature where S1 is saturated and S3 is enhanced.

and the Boltzmann populations change. The dependency of amplitude on power shows that this signal is difficult to saturate even at He temperatures. Secondly, S1, which saturates very easily at these temperatures, decreases dramatically with increasing power. In addition, at 3.5 K and high microwave power, a weak broad inflection of unknown origin also is observed[5] (see arrows in Figure 1, parts B and C) in the derivative absorption spectrum in the $g = 2$ region.

At even lower temperatures ($T \sim 2$ K) the EPR signals of most metalloproteins saturate readily and the electron spin–lattice relaxation time (T_1) and the field modulation frequency ($\omega_m = 2\pi\nu_m = 2\pi \times 10^5\ s^{-1}$) commonly obey the condition $\omega_m > 1/T_1$. Under this condition, when the saturated EPR signal of a frozen-solution sample is phase-sensitive detected with a spectrometer tuned to the dispersion mode, the response approximates to the undifferentiated absorption envelope.[7] Detection of EPR signals by this passage technique has the advantage that it enhances the detection of very broad and poorly resolved spectra and discriminates among overlapping signals that saturate at different levels of incident microwave power.

Figure 2 presents the passage signals of the VFe protein obtained as described above. With 0.02 mW of incident power, an absorption-like axial signal centered at $g \sim 2$, with width of ~250 G, dominates the EPR response; this corresponds to S1 in Figure 1. S2 is also weakly visible in this spectrum at ~1100 G.

A 10-fold increase of power to 0.2 mW has no substantial effect on the already-saturated S1 resonance but begins to bring the S2 signal into passage, as can be seen in the region with $H \geq 1100$ G. However, the major effect of the power increase is to disclose a new signal (S3), whose broad, structureless absorption envelope

† Louisiana State University.
‡ Northwestern University.

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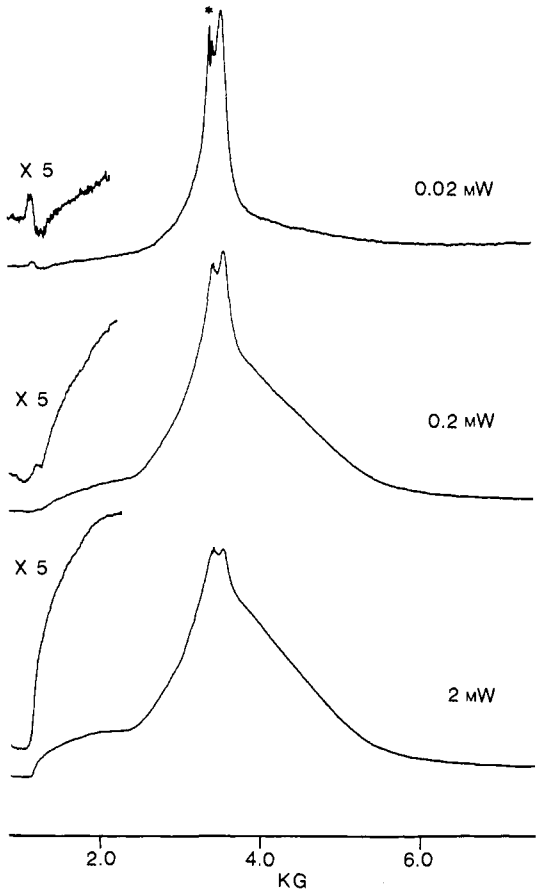

Figure 2. Dispersion spectra of VFe protein at 2.0 K showing the effect of power. Conditions: protein concentration, 60 mg mL^{-1}; sp act., 230 nmol of C_2H_2 reduced min^{-1} (mg of protein)$^{-1}$; modulation amplitude, 2 G; 100-kHz field modulation ($H_{mod} \sim 8$ G); microwave power as indicated; microwave frequency, 9.53 GHz. Major absorption about 3500 G in top spectrum is due to S1 while signal at $H \geq 1100$ G originates from S2. Broad signal between ~2800 G and ~5500 G that grows with increasing power is due to S3. The symbol (*) indicates a cavity impurity.

extends from ~2800 G to ~5500 G, with a peak near ~3500 G. No analogous signal is observed in the dispersion spectrum of the MoFe protein of conventional nitrogenase. Finally, with the power increased to 2 mW, all resonances are in passage. S1 still is present, but no longer is as evident because S3 has increased in strength and clearly has a larger integrated area. S2 now is fully absorption-like and underlies S1 and S3; it also has a large integrated area, although this is not so obvious since its spectrum is very broad, especially in the high-field region, possibly due to g-strain effects. It is interesting to note that the new signal, S3, is not present in the dispersion spectrum of Av1′ under all conditions. In particular, the spectrum of thionine oxidized Av1′, a state in which it has been shown that each of the protein's paramagnetic clusters is one-electron-oxidized,[8,9] no longer exhibits S3.

The past inability to detect S3 in the derivative absorption mode of Av1′ is due to its nonclassical line shape. Typically the absorption signal of the powder spectrum of a paramagnetic species exhibits well-defined high- and low-field shoulders easily detected in the derivative presentation. Although g-strain effects sometimes broaden the high-field shoulder beyond detection,[10,11] the low-field shoulder normally remains observable (as in the case of S2). Figure 2 shows, however, that neither edge of the S3 signal has a well-defined shoulder. This situation is not unique to S3 and has been observed in the spectra of at least two other metalloproteins, both of which involve spin-coupled centers. Horseradish peroxidase compound 1 exhibits a spectrum[12] similar to that of S3 extending from ~2400 G to ~6000 G. This spectrum[12,13] arises from the spin coupling of a porphyrin free radical to an even spin ($S = 1$) Fe^{4+} ion; the broadening of the spectrum is hypothesized to originate from a normal distribution of J coupling constants.

A similar situation exists for the reduced primary electron acceptor in the reaction-center protein of photosynthetic bacteria.[14] Here a paramagnetic ubisemiquinone anion free radical is coupled to Fe^{2+} in an $S = 2$ state. The broadened spectrum of this center results from the overlap of ground- and excited-state signals, extends from ~1200 G to ~8000 G, and again, is best simulated by using a spread of J coupling constants.

Thus, the passage EPR technique demonstrates that the paramagnetism of the as-isolated VFe protein of vanadium nitrogenase from *A. vinelandii* comprises three components, not two, and the major components appear to be S2 and S3. Unfortunately, because S2 and S3 strongly overlap and we cannot observe the full S2 signal, the absolute integrated area of each cannot be determined. Although the origins of each of the three signals in the spectrum of the VFe protein are as yet unknown, it can be stated that S1 and S2 typify spectra of metalloprotein metal clusters, and the spectrum of S3 suggests that it may originate from a paramagnetic site coupled to a metal center.

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New Azasilatranes: Sterically Induced Transannular Bond Weakening and Cleavage

Dietrich Gudat, Lee M. Daniels, and John G. Verkade*

Gilman Hall, Iowa State University
Ames, Iowa 50011

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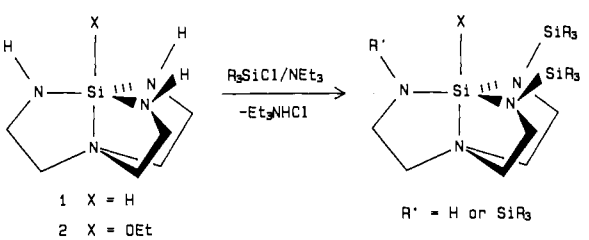
Recently we reported that azasilatranes **1** and **2** retain their structural integrity after undergoing di- or trisubstitution reactions on the equatorial NH functionalities with silyl groups of varying bulk (reaction 1).[1] Here we report that the greatly augmented



(1)

steric encumbrances resulting from the stepwise substitution of

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