THE POSSIBLE FAILURE OF ^{15}N TO AFFECT THE ESR SPECTRUM OF THE NON-HEME IRON PROTEINS OF *AZOTOBACTER VINELANDII*.*

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Asymmetrical electron spin resonance (ESR) spectra have been observed at low temperature with a variety of non-heme iron proteins under reducing conditions (1-3). Isotope substitution experiments involving ^{57}Fe (4-6) and ^{33}S (7,8) show that the ESR spectra of these proteins are due to iron in an asymmetrical ligand field and that one or more sulfur atoms serve as ligands. These considerations and the fact that the g values lie close to 2 indicate that the paramagnetic complexes resemble spin 1/2 systems and that the unpaired electrons reside in orbits having considerable s character (9,10). The requirement for reducing conditions and studies of model systems (11-13) suggest that the iron has a nominal oxidation state of Fe(I).

Whether other nucleophilic atoms, such as O or N, also are ligands of iron in these proteins is unknown. As reported here, the substitution of ^{15}N ($I = 1/2$, $\mu = -0.283$) for ^{14}N ($I = 1$, $\mu = 0.404$) in *Azotobacter vinelandii* does not seem to affect the ESR spectrum of the non-heme iron proteins.

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Experimental: A.vinelandii (strain OP, ATCC 13705) was grown at 30° in pressure vessels agitated by a rotary shaker. The nitrogen-free medium used was very similar to that used previously (7). The pressure vessels containing medium were evacuated on an oil pump for 30 min. after sterilization. Nitrogen ($^{14}N_2$ or $^{15}N_2$) was admitted to 0.7 - 0.8 atm., inoculum was added, and oxygen was admitted to provide a total pressure of 1.3 atm. Oxygen was supplied to maintain the total pressure at 1.3 - 1.5 atm. during growth of the culture, and NaOH was added to maintain a pH of 6.1 - 6.5. Yields were about 1 g packed cells/liter for cells grown under $^{15}N_2$ or $^{14}N_2$ in identical parallel experiments. The cells were harvested at 0° as quickly as possible and washed with 0.1 M phosphate buffer containing 20 mM EDTA, pH 7. The EDTA assisted in removing traces of Mn(II) which interfered with ESR observations. Cells subjected to isotope analysis were washed with 0.1 M NaCl and lyophylized.

Results: The content of ^{15}N in nitrogen gas was determined using an AEI-MS12 mass spectrometer: 97 atoms % initially; 94 atoms % after inoculation; 87 atoms %, final. Nitrogen gas derived from lyophylized cells by the Dumas method (14) contained 87 to 91 atoms % ^{15}N. The results are believed to be accurate to $\pm$ 2 atoms %. Additional evidence for ^{15}N enrichment is provided by the fact that the line width of the ESR signals from A. vinelandii at room temperature decreased in cells grown under $^{15}N_2$. The signals are presumed to be due mainly to flavin radicals. The line width ratio (^{14}N cells/^{15}N cells) at an enrichment of 85 to 90 atoms % ^{15}N extrapolated to 1.21 $\pm$ 0.03 at zero modulation amplitude. The nominal line width in the case of cells grown under $^{14}N_2$ was 21 gauss.

The non-heme iron spectra of A. vinelandii grown under $^{15}N_2$ (Fig. 1) were not significantly different by inspection from the spectra of cells grown under $^{14}N_2$. Analysis of the intervals A, B, C, and D of Fig. 1, based on 8 to 15 pairs of spectra, gave the following interval ratios (^{14}N cells/^{15}N cells): A, 1.023 $\pm$ 0.020; B, 0.999 $\pm$ 0.007; C, 0.997 $\pm$ 0.005; D, 1.041 $\pm$ 0.056. The values are the ratios of the means of the intervals, and the errors

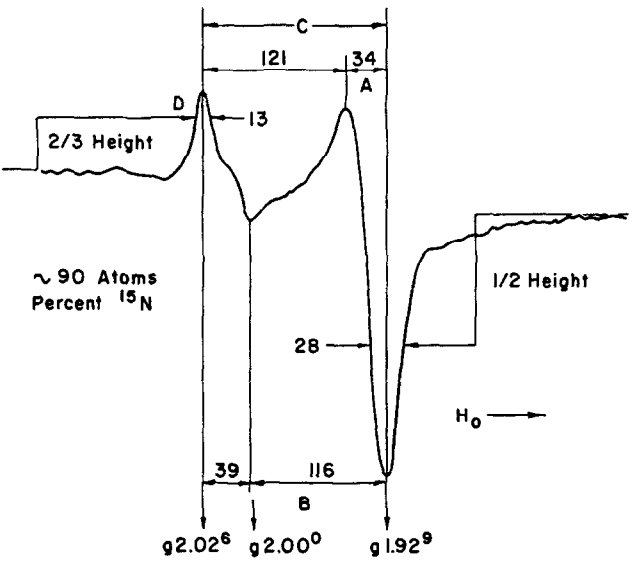

Fig. 1. ESR spectrum of A. vinelandii enriched to about 90 atoms % ^{15}N. The intervals indicated have the units of gauss, and those labeled A, B, C, and D were measured for the analysis described in the text. Instrument conditions: 8.810 GHz at 40 milliwatts incident on the critically coupled cavity; field modulation, 6 gauss peak-to-peak amplitude at 6085 Hz; H_o sweep rate, 2 gauss/sec.; time constant, 1.4 sec.; sample temperature, 77°K; signal to noise ratio ~70.

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were computed from the fractional standard deviations of the means of the intervals (15). The errors correspond to a standard deviation of about ± 0.4 gauss in the means of each of the four sets of intervals considered.

If the superhyperfine interaction were as strong on N as on S (7), we could have observed a decrease in the width of one or both lines of the non-heme iron spectra at an enrichment of 90 atoms % ^{15}N. The present results are probably negative, since no interval ratio deviates from 1 by two standard deviations of the mean. Either N is not a ligand of iron in the bulk of the non-heme iron proteins of A. vinelandii, or, if it is, superhyperfine coupling between Fe and N is weaker than that between Fe and S.

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