New Biological Paramagnetic Center: Octahedrally Coordinated Nickel(III) in the Methanogenic Bacteria

Abstract. Methanobacterium bryantii *was grown in medium supplemented with nickel-61, an isotope with a nuclear spin of 3/2. The appearance of nuclear hyperfine structure in the electron paramagnetic resonance spectrum of a cell extract identified a previously observed signal as nickel(III) in an environment of octahedral coordination with rhombic distortions.*

The methanogens are a group of organisms that constitute the sole biological source of methane. These organisms have been proposed to represent a separate line of prokaryotic descent termed archaebacteria (*1*). Although the methanogens are a very diverse group, a common characteristic is their ability to derive all cellular energy requirements from the reduction of carbon dioxide by hydrogen to form methane

$$4H_2 + CO_2 \rightarrow 2H_2O + CH_4$$

The mechanism whereby the organism derives useful energy from this reaction is unknown, although there is evidence that a transmembrane electrochemical ion gradient is involved (*2*). Methanogens apparently contain no quinones or hemes (*3*). To detect the presence of membrane-bound electron transfer components, I examined cell extracts by the use of low-temperature electron paramagnetic resonance (EPR) spectroscopy (*4*). In addition to several nonheme iron–sulfur centers, an unusual signal was present; this center was tentatively attributed to nickel(III), largely by a process of elimination of other known biological centers. I now report positive identification of this center as octahedrally complexed nickel(III) in an environment of rhombic symmetry. This represents a new biological paramagnetic center.

The lower trace of Fig. 1A shows the two low-field features of the signal present in *Methanobacterium bryantii*. These sharp features are present at g = 2.30 and g = 2.23. No nuclear hyperfine interaction is observed; this is consistent with the assignment of nickel, since the two most abundant isotopes of nickel have no nuclear spin. However, ^{61}Ni has a nuclear spin of 3/2, with a value for nuclear magnetic moment of −0.7487 magneton. The upper trace of Fig. 1A shows the spectrum of an identically prepared extract of bacteria which have been grown in medium supplemented with 88.8 percent enriched ^{61}Ni. A significant broadening of the g = 2.3 feature, indicative of unresolved hyperfine structure, occurs because the splittings are comparable in magnitude to the line width of the signal. The g = 2.23 feature is significantly distorted, and this is also indicative of unresolved hyperfine structure. The value for the hyperfine splitting of nickel-61 in other systems has been reported to be 5 to 15 gauss (*5*). Such a splitting may well be unresolved in a signal such as that of Fig. 1A (lower trace) with a width of approximately 15 gauss at half-height.

The upper trace of Fig. 1B shows an expanded-scale scan of the g = 2.02 feature of the native signal. The lower trace shows the four well-resolved hyperfine features of the ^{61}Ni sample, being equally split on the two sides of the unenriched sample. The magnitude of the splittings is approximately 20 gauss.

Double integration of this signal yields a value for concentration of spins of 0.52 nmole per milligram of protein. This compares favorably with the total amount of nickel present, determined by flameless atomic absorption spectrophotometry to be 0.43 nmole per milligram of protein (*6*). It is, however, not necessarily true that the nickel(III) oxidation state is functional physiologically because the extracts used here have been exposed to air.

These results, coupled with the previous considerations (*4*), provide strong evidence that the species exhibiting this signal is nickel(III) in an environment of rhombically distorted octahedral coordination. The species is low-spin d^7 with the unpaired electron residing in an orbital of primarily d_{z^2} character.

Because of the sensitivity of the EPR signal to ligand identity and geometry, this technique may prove to be a valuable probe for examining the role of this center in vivo in the metabolism of methanogens. Signals similar to the one in *M. bryantii* are present in at least two other species of methanogen (*7*). Nickel is a growth requirement for at least some species (*8*) and has been shown to be present in a purified hydrogenase from *M. thermoautotrophicum* (*9*). The characteristics of the species reported here are consistent with the possibility that this signal is from factor F_{430}, a chromophoric factor isolated from extracts of methanogens, which contains nickel probably present in a tetrapyrrole structure (*10*). However, repeated attempts to detect the presence of factor F_{430} in preparations exhibiting the EPR signal have been unsuccessful (*7*).

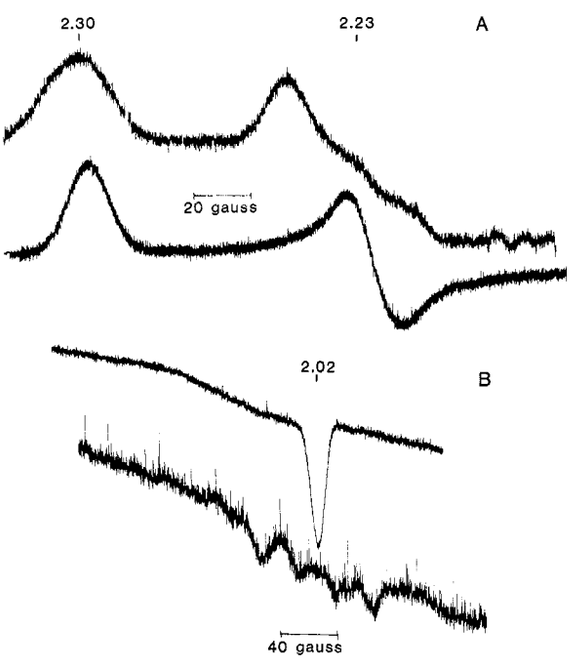

Fig. 1. Electron paramagnetic resonance spectra of preparations of *Methanobacterium bryantii*. Cells were grown on medium supplemented with 88.8 percent enriched ^{61}Ni (obtained from Oak Ridge National Laboratories); extracts were prepared as described in (*4*). Instrument settings: 9.038-GHz microwave frequency, 100-kHz modulation frequency. 77 K. (A) Lower trace, natural isotope; upper trace, ^{61}Ni-enriched (5-gauss modulation amplitude for both). (B) Upper trace, natural isotope; lower trace, ^{61}Ni-enriched (10-gauss modulation amplitude for both).

JACK R. LANCASTER, JR.
Department of Chemistry and Biochemistry, Utah State University, Logan 84322

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Proton Nuclear Magnetic Resonance of Intact Friend Leukemia Cells: Phosphorylcholine Increase During Differentiation

Abstract. *Proton nuclear magnetic resonance of intact Friend leukemia cells was used to analyze their erythroid-like differentiation. The technique, which requires only 10^8 to 10^9 cells and approximately 2 minutes for acquisition of each spectrum, demonstrated the occurrence of many signal changes during differentiation. With cell extracts, 64 signals were assigned to 12 amino acids and 19 other intermediary metabolites, and a dramatic signal change was attributed to a fourfold increase in cytoplasmic phosphorylcholines.*

Noninvasive study of whole cell biochemistry has been pioneered through investigations of energy metabolism by ^{31}P nuclear magnetic resonance (NMR) spectrometry of adenosine triphosphate (*1, 2*). Addition of ^{13}C-enriched metabolites to cell suspensions has demonstrated the potential of ^{13}C NMR monitoring of metabolic processes involving these compounds (*2, 3*). Proton NMR can detect a wide range of compounds simultaneously and at higher sensitivity than ^{13}C or ^{31}P NMR (*4, 5*). It also has the advantage that isotopic substitution of ^{1}H for ^{2}H is readily observed (*6*). Moreover, this nucleus is suitable for studies of membrane transport by spin echo methods (*7*). In this report we describe the use of ^{1}H NMR in an investigation of the erythroid-like differentiation of Friend leukemia cells (FLC's) induced by dimethyl sulfoxide (DMSO). Many compounds of the cells' intermediary metabolism can be rapidly monitored by whole cell ^{1}H NMR. The number of cells required is small enough for the technique to be applicable, in general, to mammalian cells grown in culture.

Friend leukemia cells (*8*), cultured in suspension in Eagle's minimal essential medium with 10 percent calf serum, were induced into erythroid-like differentiation with concomitant hemoglobin synthesis by addition of DMSO (2 percent by volume). The cultures were monitored as previously described (*9*). Globin production was detected after 40 hours, large numbers of hemoglobin-containing cells were seen after 50 hours, and 85 to 90 percent of all cells were producing hemoglobin by 90 hours after addition of DMSO. Cells were collected at various times by centrifugation and washed twice with medium. Washed cells were resuspended in medium to a concentration of 7×10^8 cells in 0.5 ml and placed in a 5-mm NMR tube. Proton NMR was recorded on a 470-MHz instrument with an Oxford Instrument Company magnet and a Nicolet 1180 computer. Temperature was controlled at 37° ± 1°C. Signals from the medium itself were negligible except for those of glutamine and glucose, which occur in concentrations greater than 1 m*M*. Cell viability was checked after each NMR experiment by using trypan blue exclusion and found to be 90 percent or better. Other experimental details including preparation of the cytoplasmic extracts are given in the figure legends.

A spin echo (90°–τ–180°–τ) pulse sequence was used to detect, preferential-

Table 1. Changes in signal intensity during FLC differentiation. Areas of signals from spin echo spectra (τ = 60 msec) of FLC's at various stages of differentiation (0, 9, 34, 60, 88, and 105 hours after DMSO addition) were normalized to the signal at 2.12 ppm, which remained relatively constant over the entire differentiation period. Observed changes in the areas of some of the resonances numbered in Fig. 1 are shown here. Undifferentiated FLC's for 0-hour spectra were obtained from cultures of high density, 2×10^6 cells per milliliter. Thus spectra of these cells act as a control for the possible effects of increasing cell density during differentiation in which cultures undergo approximately two doublings.

Signal	Chemical shift (ppm)	Relative area during differentiation of FLC's at time (hours)					
		0	9	34	60	88	105
7	3.55	2	2	3	3	9	10
8	3.45	5	6	8	8	12	20
9, 10	3.20	24	43	46	45	73	90
16	2.35	16	12	12	14	18	22
21	1.47	1	1	2	1	2	2

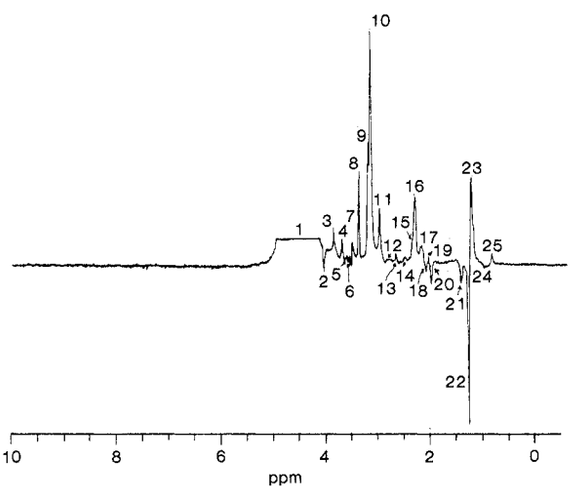

Fig. 1. Proton NMR spectrum of intact FLC's. Cells were harvested at various times after induction of erythroid differentiation. For each time period 7×10^8 cells, suspended in medium to a volume of 0.5 ml, were placed in a 5-mm NMR tube. A spin echo pulse sequence was used to collect the data at 470 MHz and 37°C. Accumulation time was 2.5 minutes (8000 points; 112 scans). The spectrum shown is of FLC's after 60 hours of differentiation and taken with τ = 60 msec. The most prominent signals are numbered; the signal from H_2O (signal 1) was truncated during plotting. The chemical shift scale is referred to external sodium 3-trimethylsilylpropionate (TSP) in a capillary. Signal assignments were made as described in the text and are as follows: 1, H_2O; 2, lactate; 3, phosphocreatine; 4, amino acid Cα; 5 and 6, glycerol and sugars; 7, glycerol; 8, unassigned; 9, phosphorylcholine (glycerophosphorylcholine); 10, choline, 11, phosphocreatine; 12, aspartate (also between signals 13 and 14); 13, citrate; 14, citrate; 15, pyruvate and succinate; 16, glutamate and proline; 17, unassigned; 18, glutamate; 19, proline; 20, isoleucine and proline; 21, alanine; 22, lactate; 23, triglycerides; 24, valine, isoleucine, and leucine; and 25, leucine and isoleucine.

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