ESR study of the primary electron donor in highly ^{13}C-enriched *Chlorobium limicola* f. *thiosulfatophilum*

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The photo-induced electron spin resonance signal of the primary donor P840$^+$ in unit membrane fractions of the title photosynthetic bacterium was measured. Bacteria containing natural isotopic abundances and 82% ^{13}C-enrichment were examined. Second moment analysis of the lineshape of the ^{13}C-enriched P840$^+$ signal shows that P840$^+$ consists of two bacteriochlorophyll *a*-type macrocycles. The data thus obtained are independent of difficulties in interpreting either peak-to-peak linewidths or ENDOR data with respect to intermolecular spin delocalization.

ESR *Reaction center* *Bacteriochlorophyll* Chlorobium limicola *Carbon 13 enrichment*

1. INTRODUCTION

Several magnetic resonance studies of the photo-oxidized primary donors of photosynthetic organisms have focused on determining the number of chlorophyll molecules interacting in the oxidized donor [1–4]. The exact number of molecules over which the resulting cation radical is delocalized becomes an important consideration in determining the mechanism of photosynthetic electron transfer. Early work on purple photosynthetic bacteria showed that the oxidized primary donor P870$^+$ exhibits a single gaussian ESR line with a linewidth that is narrowed relative to that of bacteriochlorophyll-*a*$^+$ (BChl-*a*$^+$) in vitro [1]. However, since line narrowing is insufficient evidence to prove intermolecular delocalization, electron–nuclear double resonance (ENDOR) studies on these organisms were done [2,3]. For *Rhodospirillum rubrum* the measured hyperfine splittings in the solid state at low temperature are ~½ those of BChl-*a*$^+$. It is not widely recognized that the ENDOR evidence for the interaction of 2 BChl-*a* molecules is itself insufficient without an assignment of the ENDOR transitions to specific protons in the BChl-*a* molecule. This assignment in *R. rubrum* can be made by selective biosynthetic deuteration of the chromophores in vivo. Thus, the spin can be accounted for completely in the oxidized primary donor. This type of evidence simply does not exist for the other photosynthetic organisms.

To remedy this situation we have developed an alternate approach to counting the number of chlorophyll molecules involved in the oxidized primary donors of photosynthetic organisms [5,6]. In organisms with natural isotopic abundances the ESR linewidths exhibited by the oxidized donors are dominated by β proton hyperfine interactions. Ligation changes at the central magnesium atom [7] and proton shifts in ring V [5] of chlorophylls result in spin density distributions in which β proton hyperfine splittings are greatly diminished. The result is narrowing of the ESR line even though no intermolecular spin delocalization occurs. Our technique involves the use of highly ^{13}C-enriched organisms. In the highly ^{13}C-enriched oxidized primary donor every carbon atom over which spin may be delocalized contributes to the ESR lineshape. Second moment analysis [8] of the ESR line observed for the ^{13}C-enriched organism and a comparison of the results with those obtained for the corresponding ^{13}C-enriched monomeric chlorophyll cation radical in vitro directly yield the number of chlorophylls over which the spin is delocalized.

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2. MATERIALS AND METHODS

Chlorobium limicola f. *thiosulfatophilum* was enriched in ^{13}C by using 90% ^{13}C Na_2CO_3 as the carbon source in the growth medium [9]. About 8 g wet packed cells were collected from 41 culture. A small sample of cells was dehydrated in vacuo and analyzed for ^{13}C content by pyrolysis followed by mass spectroscopy of CO_2. The cells were 82% enriched in ^{13}C. The wet packed cells were used to prepare Olson's complex I (unit membrane vesicles) [9,10]. The isolated subcellular fraction was substantially free of chlorobium chlorophylls and pheophytins as indicated by the appropriate optical absorbance ratios, $A_{810}/A_{760} = 8$ and $A_{810}/A_{674} = 1.5$. Complex I was also prepared from cells having natural isotopic abundances.

ESR spectra were obtained with a Varian E-9 system. Low temperatures were provided by an Air Products helium cryostat with an Oxford Instruments temperature controller. All spectra reported here were obtained at 35 ± 1 K. Microwave power was maintained below 0.1 mW and field modulation was maintained below 1.0 G to minimize instrumental line broadening. Samples were irradiated with a 300 W Eimac Xe arc lamp equipped with a heat absorbing filter and a cutoff filter passing wavelengths >600 nm. Light-on and light-off spectra were recorded. Illustrated spectra are the average of 64 field scans were recorded and stored with a DEC LSI-11 computer system.

3. RESULTS

Fig. 1 shows the light-minus-dark ESR signals obtained from P840$^+$ in complex I preparations which possess either natural abundance ^{13}C or 82% enrichtment in ^{13}C. The 9.3 G linewidth and Gaussian lineshape of the natural abundance P840$^+$ spectrum is similar to that obtained in [10]. ^{13}C-Enrichment increases the peak-to-peak linewidth to 12.1 G and results in a non-Gaussian lineshape. The second moment of each ESR line was calculated by double integration of the computer-averaged data. The measured second moments are 20 G^2 for natural abundance P840$^+$ and 85 G^2 for 82% ^{13}C-enriched P840$^+$. The level of uncertainty in the second moment calculations for the noise level encountered in these experiments is ±15%.

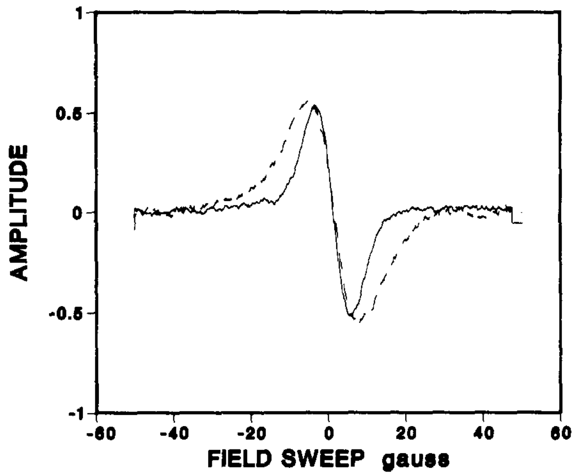

Fig. 1. Light-minus-dark ESR signals of complex I prepared from natural abundance (——) and 82% ^{13}C-enriched (---) *C. limicola* f. *thiosulfatophilum*. The center of each ESR signal occurs at $g = 2.0025 \pm 0.0001$.

4. DISCUSSION

The increase in linewidth and the change to non-Gaussian lineshape upon ^{13}C-enrichment is consistent with observations of P870$^+$ and P700$^+$ in other photosynthetic organisms [6]. The linewidth of the natural abundance P840$^+$ signal approaches the value predicted by the Norris $1/\sqrt{2}$ relationship relative to the 12.8 G linewidth of natural abundance BChl-a^+ in vitro [1]. However, as noted above, this observation is itself insufficient to determine the number of BChl *a* molecules over which the spin is delocalized in P840$^+$.

Since contributions to the observed second moment of the ESR line that are due to the hyperfine interactions of ^1H and ^{13}C nuclei are rigorously additive [8], we may use the second moment data of the natural abundance P840$^+$ signal as a good measure of the ^1H contribution to the measured second moment of the ^{13}C-enriched P840$^+$ signal. Thus, the ^{13}C contribution alone is simply $85\,G^2 - 20\,G^2 = 65\,G^2$. This number should be compared with the second moment for ^{13}C in 82% ^{13}C-enriched BChl-a^+ in vitro. This number is 117 G^2 [6]. Thus, the value of the ^{13}C second moment for P840$^+$ is ~½ that of the corresponding BChl *a* cation in vitro.

Since the ^{13}C carbon atoms in the π cation framework of the BChl *a* molecule or molecules

Table 1

Spin delocalization within the primary donors of photosynthetic organisms

Organism	Oxidized donor	No. Chl over which spin is delocalized	Method	[Ref.]
Rhodospirillum rubrum	P870$^+$	2	ENDOR	[2,3]
			^{13}C second moment	[6]
Rhodopseudomonas sphaeroides	P870$^+$	2	ENDOR	[4]
Chlorobium limicola f. *thiosulfatophilum*	P840$^+$	2	^{13}C second moment	[here]
Scenedesmus obliquus	P700$^+$	1	^{13}C second moment	[5,6]
			ENDOR	[11]

comprising P840$^+$ monitor the entire spin irrespective of its detailed distribution, our data is consistent with delocalization of the spin over two BChl *a* molecules. In this regard P840$^+$ bears a strong resemblance to P870$^+$ in purple photosynthetic bacteria.

Since our experiments were performed at cryogenic temperatures there always exists the question of whether the detailed spin distribution within P840$^+$ is different at ambient temperature. Addressing a similar problem, the liquid solution ENDOR spectrum of P870$^+$ was determined in reaction centers from the R-26 mutant of *Rhodopseudomonas sphaeroides* [4]. While the detailed spin distribution changes somewhat as a function of temperature, the data remain consistent with delocalization of the spin over two BChl-*a* molecules near ambient temperature. Similarly, their data for P700$^+$ in photosystem I near ambient temperature agrees with our independent determination of the monomeric nature of P700$^+$ using the ^{13}C-enrichment–second moment technique [11]. Thus, we expect our results to remain unchanged at ambient temperature.

A summary of results on spin delocalization within the oxidized primary donors of photosynthetic organisms is given in table 1.

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