SPIN COUPLING BETWEEN ELECTRON CARRIERS IN THE DEHYDROGENASE SEGMENTS OF THE RESPIRATORY CHAIN

John C. Salerno and Tomoko Ohnishi

Department of Biochemistry and Biophysics
School of Medicine
University of Pennsylvania
Philadelphia, PA 19174

Joyce Lim, William R. Widger, and T.E. King

Department of Chemistry and Laboratory for Bioenergetics
State University of New York at Albany
Albany, New York 12222

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SUMMARY

The binuclear iron sulfur clusters S-1 and S-2 in all succinate dehydrogenase preparations are spin coupled, which is reflected in the saturation behavior of S-1 signal in the presence of S-2 spins and in lineshape modification of dithionite reduced enzymes at low temperatures. An additional EPR signal has been detected at approximately half the applied field of the 'g=1.94' signal corresponding to 'ΔMs=2' transition. A similar signal has been observed in Complex I preparations; one of the interacting species is iron sulfur Center N-3 (g_x=1.86).

INTRODUCTION

EPR signals from the iron-sulfur clusters have been reported in succinate dehydrogenase (1-4) and NADH dehydrogenase (5-7) by a number of workers. Recently, we proposed that the binuclear iron-sulfur clusters in soluble succinate dehydrogenase (Centers S-1 and S-2) were spin coupled on the basis of the low temperature broadening or splitting observable (under non-power saturated conditions) in the EPR spectra of the reconstitutively active or inactive enzymes, respectively; and the desaturation of EPR signals arising from Center S-1 observable on the reduction of Center S-2 over a wide range of temperature both in active and inactive preparations (2).

Mathews et al (8) demonstrated that the complex EPR spectra of bacterial (eight iron) ferredoxins arise from interaction between two nearly identical

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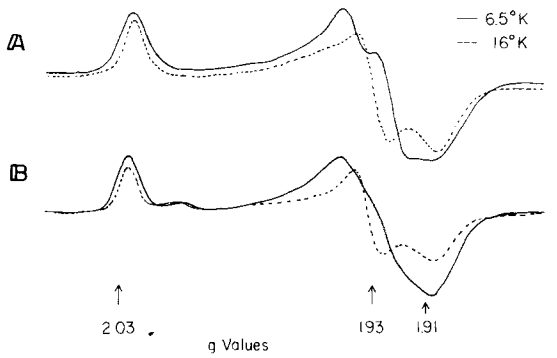

Figure 1. X-band EPR spectra of succinate dehydrogenase. Solid line, 6.5°K; dotted line, 16°K. Modulation amplitude, 5 gauss. Modulation frequency, 100 KHz. Microwave power, 1 mW. Microwave frequency, 9.14 GHz.

A) B-SDH (39 mg protein/ml; 2 nmoles flavin/mg protein) reduced with 10 mM succinate

B) BS-SDH (20 mg protein/ml, 4 nmoles flavin/mg protein) reduced with dithionite

tetranuclear iron-sulfur clusters. They observed an EPR signal in the fully reduced enzyme corresponding to a '$\Delta M_s=2$' transition within the triplet formed by the coupled $S=\frac{1}{2}$ paramagnets. This 'half field resonance' has a transition probability proportional to $(D/H)^2$; thus, as the strength of the coupling decreases, its intensity falls rapidly to zero (9).

In this investigation, similar half field signals were observed in both succinate dehydrogenase and NADH-UQ reductase (Complex I) implying the existence of spin coupling in both enzymes.

MATERIALS AND METHODS

Reconstitutively active and inactive succinate dehydrogenase (BS-SDH and B-SDH) were prepared as described previously (2). Complex I was prepared by a previously described procedure (10).

EPR measurements were performed on a Varian E-109 microwave spectrometer. Temperature control was obtained by the use of a flowing helium cryostat (Air Products Model LTD-3-110). Temperature was measured with a carbon resistor located below the sample.

RESULTS AND DISCUSSION

The spectrum of B-SDH at low temperatures, shown in figure 1a (solid line), exhibits splitting of the central (g=1.94) peak concomitant with broadening

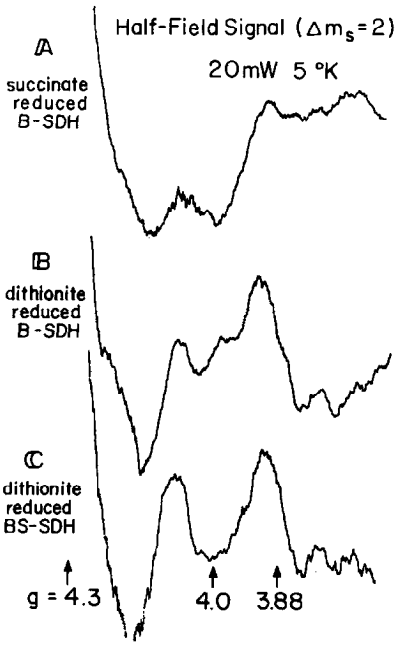

Figure 2. X-band EPR spectra of succinate dehydrogenase in the half field region. Modulation amplitude, 12.5 gauss. Modulation frequency, 100 KHz. Microwave power, 20 mW. Microwave frequency, 9.14 GHz. Time constant, 1s; temperature, 5°K.

A) B-SDH (39 mg protein/ml, 2 nmoles flavin/mg protein) reduced with 10 mM succinate

B) B-SDH reduced with dithionite

C) BS-SDH (20 mg protein/ml, 4 nmoles flavin/mg protein) reduced with dithionite

of g_x and g_z peaks which disappears upon raising the temperature (dotted line). Unsaturated EPR spectra of BS-SDH broaden as the temperature is lowered, (Figure 1b) but even at 4.5°K no splitting is resolved (2). Desaturation of Center S-1 is observed upon reduction of Center S-2 in both enzymes in certain ranges of temperature and power.

Figure 2a shows the EPR spectra in the half field region of B-SDH reduced with succinate. The large signal on the left is the so-called 'g=4.3 signal' from ferric iron (11); no other prominent resonances were observed. Upon

reduction with dithionite, a relatively weak, isotropic signal near g=3.88 was observed in both B-SDH (figure 2b) and BS-SDH (figure 2c). The nearly identical intensities of the two enzymes imply that the spin coupling is of nearly identica magnitude in each, due to the strong dependence of the transition probability on the coupling strength.

The signal around g=4.05, which is fairly intense in the active enzyme (BS-SDH, figure 2c), is of unknown origin. Its nearness to the field position expected for the $\Delta M_s=2$ transition of a radical pair is probably coincidential, the signal is most likely due to a contaminant and is prominent only because the relatively small transition probability of the $\Delta M_s=2$ transition necessitates the use of unusually high gain.

At this temperature (5°K), the antiferromagnetically coupled high spin ferrous and ferric iron atoms in each binuclear cluster will form $S=\frac{1}{2}$ paramagnets, and the coupled system can be represented as a singlet and triplet. The 'g=1.94' type signals will then arise from the $\Delta M_s=1$ transitions between the $\Delta M_s=-1,0$ and $0,1$ levels and the half field signal corresponds to the $\Delta M_s=2$ transition between the $M_s = \pm 1$ levels, exactly analogous to the case of the bacterial ferredoxins (8). The low anisotropy of the '$\Delta M_s=2$' signal is not expected, since it is second order in the spin orbit coupling ($\Delta g/g$). The intensity of the $\Delta M_s=2$ transition is at least a few thousand times weaker than the $\Delta M_s=1$ transitions shown in figure 1. However, the lack of anisotropy enables it to be observed at only a few hundred times the gain. This lower transition probability also makes the use of high microwave power possible without saturation, even at 5°K.

The signal was sensitive to the angle between the applied constant magnetic field and the magnetic component of the microwave field, increasing by about 60-70% as the angle decreased from 90° to 83°.

Iron-sulfur Center N-3 in Complex I exhibits a characteristic rhombic EPR spectra ($g_z=2.035$, $g_y=1.93$, $g_x=1.86$) in the reduced form at temperatures

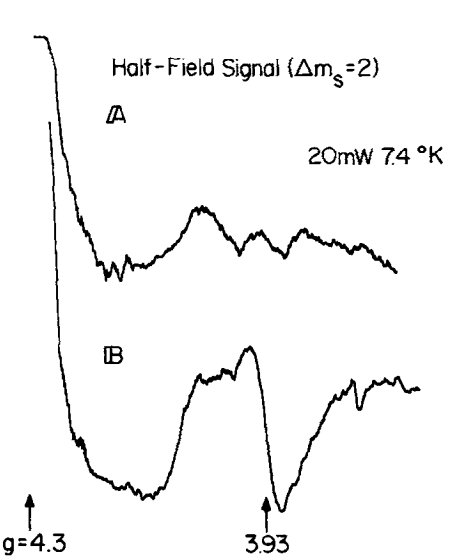

Figure 3. X-band spectra of Complex I in the half field region. EPR conditions as in Figure 1, except temperature, 7.4°K.

A) Complex I (35 mg protein/ml) was poised at -292 mV with potentiometric techniques (14, 15) in the presence of phenosafranine, methyl and benzyl viologens with dithionite as reductant

B) As in A, except E_h = -380 mV

below 20°K (12[a], 13). Potentiometric titrations have shown that the signal appears as the potential is lowered, with a half reduction potential of about -240 mV and an n value of approximately 1. Further lowering of the potential produces an interesting anomaly; the signal at g=1.86 decreases in amplitude, reaches a minimum at about -380 mV, and then increases to its maximum value[b]. Concurrently, the signal shown in figure 3 appears as the potential is lowered, reaches a maximum at -380 mV, and disappears with further lowering of the potential.

The transient nature of these phenomena suggest the possible involvement

[a] These authors (refs. 12) employ a nomenclature in which this center is designated 'N-4.'

[b] Manuscript in preparation by Ohnishi, T., Salerno, J.C., Widger, W.R., Ragan, C and King, T.E.

of a two electron carrier with a paramagnetic intermediate redox state; the observed g-value is consistent with either a free radical iron-sulfur center interaction or two strongly coupled iron-sulfur centers[c]. The governing factor in the latter case may be the sequential reduction of two single electron carriers, perhaps with the second reduction coupled to a conformational change.

Although the nature of the interaction in Complex I is not yet well understood, it is important as a potential clue to the sequence and mechanism of electron transport; it may also affect the interpretation of the heavily overlapped EPR spectra around g=2 and g=1.94 in Complex I.

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[c] The redox mediators used are one electron carriers. No correlation was observed between free radical signal of mediators and the appearance of the half field signal.

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