LOW TEMPERATURE ELECTRON PARAMAGNETIC RESONANCE STUDIES ON TWO IRON-SULFUR CENTERS IN CARDIAC SUCCINATE DEHYDROGENASE

Tomoko Ohnishi
Department of Biophysics and Physical Biochemistry
Johnson Research Foundation
University of Pennsylvania
Philadelphia, Pennsylvania 19174

Daryl B. Winter, Joyce Lim, and Tsoo E. King
Department of Chemistry
State University of New York at Albany
Albany, New York 12222

Received May 10, 1973

SUMMARY: At temperatures below 20°K, EPR signals from a new iron-sulfur center (designated here as Center S-2 or $(Fe\text{-}S)_{S-2}$) in addition to the classical "g = 1.94 signal" (designated as Center S-1 or $(Fe\text{-}S)_{S-1}$) were detected in purified, soluble succinate dehydrogenase, particulate succinate ubiquinone reductase (Complex II) and particulate succinate cytochrome c reductase from bovine heart. The measured half-reduction potential ($E_{m7.4}$) of Center S-1 was 0 ± 10 mV, while $E_{m7.4}$ of Center S-2 was -260 ± 15 mV in the membrane bound preparations. Upon solubilization of succinate dehydrogenase, the EPR behavior of Center S-2 became extremely labile similar to the characteristics of the reconstitutive activity of succinate dehydrogenase toward the rest of the respiratory chain.

It has long been suggested that succinate dehydrogenase contains at least two distinct iron-sulfur moieties (1). This suggestion is based on the measured content of 8 iron and 8 labile sulfide per enzyme molecule (2), the results of the reconstitution and other experiments (1,3) and the isolation of a proteolytically resistant iron-sulfur protein from the dehydrogenase (4). More recently the separation of an iron-sulfur protein and a flavo-iron-sulfur protein has also been reported by treating the enzyme with sodium dodecylsulfate or trichloroacetic acid (5). Multiple iron-sulfur centers have been demonstrated in the NADH-ubiquinone segment of the respiratory chain by EPR measurements at temperatures below 77°k (6,7). The half-reduction potential of these iron-sulfur centers (8,9) has been determined by the potentiometric titration procedure (10). In the present study, these experimental methods have been applied to the

succinate-UQ segment. Here we wish to report the existence of two different iron-sulfur centers in the succinate-dehydrogenase region of the respiratory chain.

MATERIALS AND METHODS

Soluble succinate dehydrogenase (11), succinate cytochrome c reductase (12), and succinate-UQ reductase (Complex II)* (13) were prepared from bovine heart by reported methods with minor modifications. All samples were kept frozen in liquid nitrogen and thawed anaerobically before the experiments. The half-reduction potentials of iron-sulfur centers were measured potentiometrically according to Dutton (10) and Wilson et al. (8). Redox titration was performed at room temperature and aliquots were transferred anaerobically to EPR sample tubes and rapidly frozen in liquid isopentane at 113°K. EPR measurements were recorded with a Varian Model E-4 spectrometer at various temperatures as reported previously (6). The temperature was monitored by an Au/Co versus Pt thermocouple placed just below EPR samples.

RESULTS AND DISCUSSION

EPR studies at 77°K (15,16) and even lower temperatures (17) of soluble succinate dehydrogenase preparations with various ratios of flavin to iron to sulfide gave no indication of the multiplicity of iron-sulfur centers in the enzyme. The only EPR active species other than the flavin radical has been widely known as "g = 1.94 signal" (cf. 18). As shown in Fig. 1A, EPR signals of soluble succinate dehydrogenase were obtained at g = 2.03, 1.94 and 1.92 at temperatures above 20°K. These

* It may be pointed out that although Complex II (13.14) serves as succinate-UQ reductase, structurally the Complex II preparation also contains phospholipid and enzymatically inactive cytochrome b and c_1. In this communication, therefore, the operational term, succinate-UQ reductase was used.

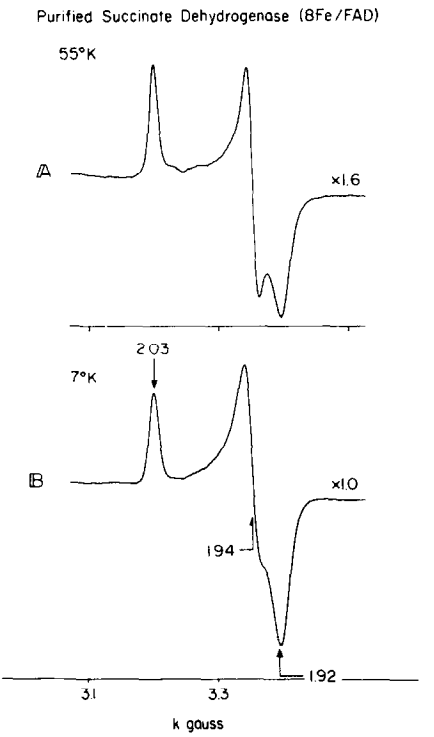
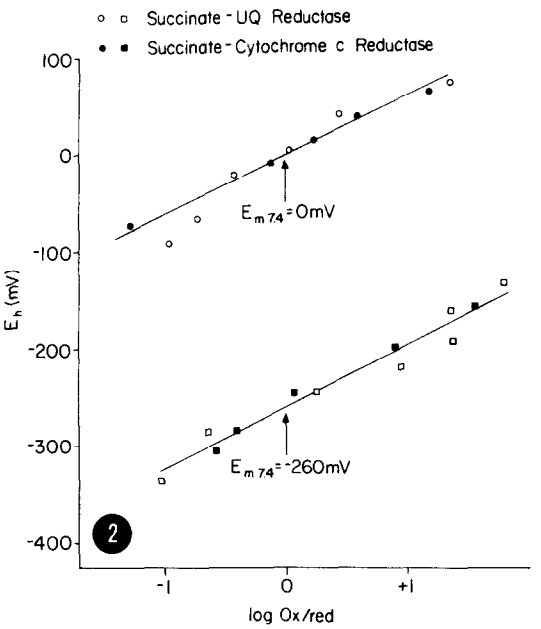


Figure 1. EPR spectra of reduced succinate dehydrogenase at 55° and 7°K. Succinate dehydrogenase (2.7 mg protein per ml) dissolved in 33 mM phosphate buffer pH 7.4, containing 6.6 mM succinate was further reduced with traces of solid dithionite. This reconstitutively active enzyme contained 8 nmoles FAD per mg protein. EPR operating conditions were: modulation amplitude, 12.5 gauss; microwave power, 20 mW, microwave frequency, 9.1 GHz; time constant 0.3 sec.; scanning rate 500 gauss per min.

signals evidently belong to the classical "g = 1.94 signal" -- designated herewith as Center S-1 or $(Fe\text{-}S)_{S-1}$. Upon lowering the temperature, the intensity of signals was enhanced and additional EPR signals emerged at the same field position but with different line-shape. These characteristics became very distinct at 7 °K as shown in Fig. 1B. These new signals are designated as Center S-2 or $(Fe\text{-}S)_{S-2}$. The presence of at least two different species of iron-sulfur centers was thus suggested in soluble succinate dehydrogenase which contains 8 non-heme iron and 8 acid labile sulfide per flavin.

Similarly two species of EPR signals were observed in the dithionite reduced succinate-UQ reductase or succinate-cytochrome c reductase. Only Center S-1 was reducible by succinate in all preparations.



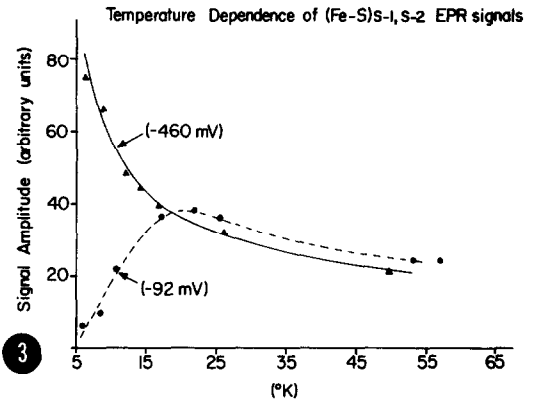


Figure 2. Plot of observed oxidation-reduction potential (E_h) as a function of $\log_{10}$ (oxidized) / (reduced) for the two iron-sulfur centers; $(Fe\text{-}S)_{S-1}$ and $(Fe\text{-}S)_{S-2}$, in the succinate-ubiquinone reductase (Complex II) and succinate-cytochrome c reductase. The redox states of $(Fe\text{-}S)_{S-1}$ and $(Fe\text{-}S)_{S-2}$ were determined from the peak to peak amplitude of the prinicipal EPR absorption bands of the reduced iron-sulfur centers. The succinate-ubiquinone and succinate-cytochrome c reductase preparations were suspended in 0.1 M phosphate buffer, pH 7.4, at protein concentration of 5.6 mg and 22.1 mg per ml, respectively. The redox mediators added were 62.5 µM phenazinemethosulfate, 62.5 µM phenazineethosulfate, 25 µM duroquinone, 6.3 µM pyocyanine, 6 µM resorufin, 25 µM 2-hydroxy-naphtoquinone, 77.5 µM phenosafranine, 94 µM benzyl viologen and 133 µM methyl viologen. The oxidation-reduction potential of the suspension was adjusted to desired values by the addition of aliquots of freshly prepared dilute solution of dithionite. EPR operating conditions were: modulation amplitude, 12.5 gauss; microwave frequency, 9.1 GHz; time constant, 0.3 sec; scanning rate, 500 gauss per min. Temperature and microwave power setting for the titration of $(Fe\text{-}S)_{S-1}$ and $(Fe\text{-}S)_{S-2}$ was 27°K and 20 mW; and 7°K and 5 mW, respectively.

Figure 3. Effect of temperature on EPR signals of two iron-sulfur centers; $(Fe\text{-}S)_{S-1}$ and $(Fe\text{-}S)_{S-2}$, in the succinate-ubiquinone reductase (Complex II) equilibrated at -92 mV and -460 mV. The conditions are detailed in the legend of Figure 2.

The half-reduction potentials at pH 7.4 ($E_{m7.4}$) of these iron-sulfur centers were determined potentiometrically using succinate-cytochrome c reductase and succinate-UQ reductase. In order to cover a wide range of oxidation-reduction potentials, nine redox mediators were added to the system as detailed in the legend of Fig. 2. EPR spectra were recorded at 27° and 7°K for the measurement of Center S-1 and Center S-2, respectively.

Peak to peak amplitude of the principal EPR absorption band was plotted as a function of the oxidation-reduction potential relative to the standard hydrogen electrode (E_h). The measured $E_{m7.4}$ value for Center S-1 was 0 ± 10 mV. This value is only slightly lower than the $E_{m7.2}$ value obtained for Center S-1 in pigeon heart mitochondria (8,9). The measured $E_{m7.4}$ value of Center S-2 was -260 ± 15 mV; it should be pointed out that this value is much lower than the value of Center S-1 and any other electron carriers located in the Site II region of the respiratory chain. An n value of 1.0 was obtained for both Centers S-1 and S-2.

The effect of temperature on EPR signals of Centers S-1 and S-2 is illustrated in more detail in Fig. 3, using succinate-UQ reductase equilibrated at redox potentials of -92 and -460 mV, respectively. At -92 mV only Center S-1 was reduced while at -460 mV both centers were in the reduced form. EPR signals from Center S-1 saturated at temperatures below 20°K, while signals from Center S-2 appeared only below 20°K. Because of the completely different behavior of the temperature dependence of these two iron-sulfur centers, EPR spectra A and B presented in Fig. 1 could be attributed solely to Center S-1 and Center S-2, respectively.

What is the functional role of Center S-2, which is not reducible with succinate? Could this low potential iron-sulfur center be an isolation artifact of these enzyme preparations? To test this possibility, EPR spectra of mitochondrial systems were examined. EPR signals arising from Center S-2 cannot be studied directly in pigeon heart or mammalian mitochondria because of overlapping signals from other iron-sulfur centers present in the Site I region. It was found, however, that the $E_{m7.2}$ values for Centers S-1 and S-2 were 0 ± 10 mV and -245 ± 15 mV, respectively, in submitochondrial particles of Saccharmoyces cerevisiae where Center S-2 signals could be measured without overlapping signals from other iron-sulfur centers (19). This fact suggests that the low $E_{m7.4}$ value of Center S-2 may not be an artifact derived from the preparation of succinate-cytochrome c reductase or

succinate-UQ reductase. Moreover, it was observed that Center S-2 was not reducible even with a low potential substrate (NADH) under anaerobic conditions in the S. cerevisiae particles. All these observations indicate that Center S-2 may not readily equilibrate with other respiratory carriers nor be directly involved in the electron transfer of the respiratory chain.

For many years it has been observed that in the visible absorption spectrum of soluble succinate dehydrogenase, succinate can only reduce in the neighborhood of 25% of the dithionite reducible spectrum (2). The finding of the second iron-sulfur center with its characteristic low $E_{m7.4}$ value may provide an explanation for this observation i.e. succinate can only reduce the first iron-sulfur center, while the addition of dithionite is able to reduce both centers, resulting in a further bleaching of the absorption spectrum.

Upon solubilization, Center S-2 became extremely unstable and a short exposure of the soluble enzyme to air inactivated this center resulting in the loss of the EPR signals. On the other hand, the same treatment caused no disappearance of the signal of Center S-1. It was found that the half-reduction potential of Center S-2 in soluble succinate dehydrogenase showed a strong tendency to decrease to a lower value, approximately -430 mV, whereas the $E_{m7.4}$ of Center S-1 remained unaltered. These observations suggest that Center S-2 may play a role in the linkage of the dehydrogenase to the rest of the respiratory chain (cf. p. 226, Ref. 2), since reconstitutive activity of succinate dehydrogenase has been found much more labile than its activity toward artificial electron carriers (1). On the other hand, the stability of Center S-1 mirrors the stability of the artificial electron transfer (for example, phenazine methosulfate) activity of the soluble enzyme. More detailed studies will be reported elsewhere (20).

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. B. Chance for his interest and encouragement, and to Dr. D.F. Wilson and Dr. J.S. Leigh for their expert suggestions. Thanks are also due to Miss S. Shiraishi for

her excellent technical assistance. This investigation was partly supported by PHS grants GM-12202, GM-16767 and HL-12576.

REFERENCES

1. T.E. King, Advances of Enzymology, 28, 155 (1966).
2. T.E. King, Biochem. Biophys. Res. Commun., 16, 511 (1964).
3. T.E. King in "Non-Heme Iron Proteins: Role in Energy Conservation", (A. San Pietro, ed.) Antioch Press, Yellow Springs, Ohio, p. 413 (1965).
4. D.F. Wilson and T.E. King, Biochim. Biophys. Acta, 131, 265 (1967).
5. K.A. Davis and Y. Hatefi, Biochemistry, 10, 2509 (1971).
6. T. Ohnishi, T. Asakura, T. Yonetani and B. Chance, J. Biol. Chem., 246, 5960 (1971).
7. N.R. Orme-Johnson, W.H. Orme-Johnson, R.E. Hansen, H. Beinert and Y. Hatefi, Biochem. Biophys. Res. Commun., 44, 4461 (1971).
8. D.F. Wilson, M. Erecińska, P.L. Dutton and T. Tzudsuki, Biochem. Biophys. Res. Commun., 41, 1273 (1970).
9. T. Ohnishi, D.F. Wilson, T. Asakura, B. Chance, Biochem. Biophys. Res. Commun., 46, 1631 (1972).
10. P.L. Dutton, Biochim. Biophys. Acta, 226, 63 (1971).
11. T.E. King, in Methods of Enzymology, 10, 322 (1967).
12. S. Takemori and T.E. King, Biochim. Biophys. Acta, 64, 192 (1962).
13. M.L. Baginsky and Y. Hatefi, J. Biol. Chem., 244, 5313 (1969).
14. D.M. Ziegler and K.A. Doeg, Arch. Biochem. Biophys., 85, 282 (1959).
15. H. Beinert and R.H. Sands, Biochem. Biophys. Res. Commun., 3, 41 (1960).
16. T.E. King, R.L. Howard and H.S. Mason, Biochem. Biophys. Res. Commun. 5, 329 (1961).
17. D. DerVartanian, C. Veeger, W.H. Orme-Johnson and H. Beinert, Biochim. Biophys. Acta, 191, 22 (1969).
18. H. Beinert, W. Heinen, and G. Palmer, Brookhaven Symposia Biol. No. 15, 229, (1962).
19. T. Ohnishi and S. Shiraishi, in preparation.
20. T. Ohnishi, D.B. Winter, J. Lim, L.Yu, C.A. Yu and T.E. King, in preparation.