AN ELECTRON SPIN RESONANCE STUDY OF SOLUBLE SUCCINIC DEHYDROGENASE*

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Several electron spin resonance (ESR) studies of heart homogenates, submitochondrial particles and intact mitochondria have been reported (Commoner and Hollocher, 1960), Beinert and Lee, 1961), but very little work has been done with purified, soluble active components. We have now examined a well-defined succinic dehydrogenase which has been shown to be active toward the cytochrome system. Our results modify and extend the earlier findings.

Materials and Methods

Soluble succinic dehydrogenase (SDH) was prepared according to a previous report (Keilin and King, 1960). ESR spectra were obtained with a Varian V-4500 x-band spectrometer using 100 kc field modulation, and modulation amplitudes of 0.2 - 8.0 gauss. Room temperature experiments were carried out with a flat quartz sample cell filled either manually by syringe or by a dual syringe driver operating through a ten-jet mixer (flow experiments). Experiments at liquid nitrogen temperature were performed with the sample in a quartz tube immersed directly in liquid nitrogen. Klystron frequencies were determined with a Hewlett-Packard K532B frequency meter and field strengths were measured with a proton resonance meter monitored by a Hewlett-Packard 524C electronic frequency counter.

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Results and Discussion

No signal corresponding to monodehydrosuccinate free radical was detected during succinate oxidation at an overall velocity of 4×10^{-4} M sec^{-1}. Typical derivative ESR spectra of SDH at room temperature (about 25°) and at liquid nitrogen temperature are shown in Fig. 1. Three absorption peaks, with g values of 1.94, 2.00 and 2.01 and an overall signal width of 218 gauss were observed at liquid nitrogen temperature. At room temperature, only a signal at g = 2.0029, with a band width of 77 gauss, was evident. All signals were abolished by heating the enzyme for 5 minutes at 60°.

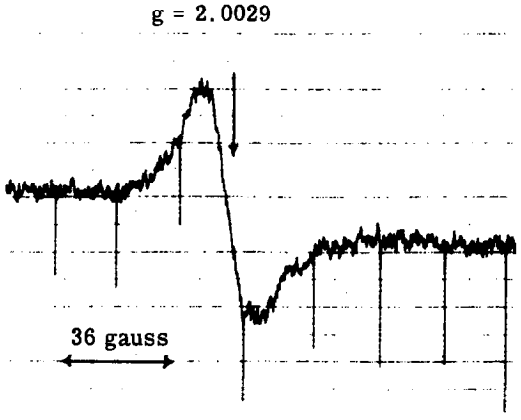

Fig. 1A

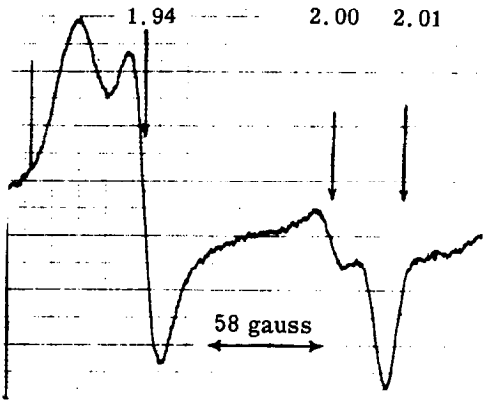

Fig. 1B

Fig. 1. ESR spectra of SDH at room temperature (A) and at liquid N_2 temperature (B). The following conditions were used: (A) modulation amplitude, 8 gauss; scan speed, 46 gauss/min.; sample cell volume, 0.075 ml. (B) modulation amplitude, 8 gauss; scan speed, 74 gauss/min.; sample cell volume, 0.2 ml. containing 2.6 mg. protein in 0.1 M phosphate buffer, pH 7.8; 5.8×10^{-2}M succinate. Enzyme activity (zero time) was 21 μmoles succinate oxidized/min/mg. enzyme protein, after reconstitution; this was 110% of the phenazine methosulfate activity.

Analysis of more than 80 ESR spectra of SDH at different times after preparation in the presence of various concentrations of substrates, selective inhibitors, and reducing agents, revealed that the value of $R_{1.94/2.01}$ was constant** (also cf. Tables I and II) in contrast to that of $R_{1.94/2.00}$

** The symbols $R_{1.94/2.00}$ and $R_{1.94/2.01}$ used in this paper denote the ratios of the signal magnitude at g = 1.94 to g = 2.00 and to g = 2.01 respectively. The statistical analysis by Dr. J. C. R. Li is acknowledged.

Table I. Effect of Succinate, Fumarate and Other Reagents on the Magnitude of ESR Signals of SDH at Liquid Nitrogen Temperature*

Exp.	Addition	Arbitrary Units g = 1.94	g = 2.00	g = 2.01	$R_{1.94/2.00}$	$R_{1.94/2.01}$
1	None	86	26	36	3.3	2.4
	Fumarate, 5.8×10^{-2} M	36	24	16	1.5	2.3
	Succinate, 1.0×10^{-5}	84	24	38	3.5	2.2
	1.0×10^{-4}	90	22	40	4.1	2.3
	1.0×10^{-3}	102	30	42	3.4	2.4
	5.8×10^{-3}	124	34	51	3.7	2.4
	5.8×10^{-2}	118	58	49	2.0	2.4
	Succinate, 2.2×10^{-2} +Fumarate, 3.6×10^{-2}	56	36	22	1.6	2.5
2	None	46	7	19	6.6	2.4
	Succinate, 5.8×10^{-2}	57	12	26	4.8	2.2
	$Na_2S_2O_4$, 5mg/ml	83	0	40	∞	2.1
	Malonate, 0.11M	24	9	10	2.7	2.4
3	None	114	18	50	6.3	2.3
	Fumarate, 5.8×10^{-6} M	110	18	45	6.1	2.4
	5.8×10^{-5}	68	20	28	3.4	2.4
	5.8×10^{-3}	56	26	22	2.2	2.5
	5.8×10^{-2}	50	46	20	1.1	2.5

*Experiments were performed under conditions as described in the legend of Fig. 1. About ten minutes were required for scanning one sample. The system in 0.2 ml. contained protein, 3.2 mg. (Exp. 1), 2.0 mg. (Exp. 2) and 3.8 mg. (Exp. 3) in 0.1 M phosphate, pH 7.8, with other components as listed. Different batches of enzyme preparation were used.

Table II. The Effect of Time on Signal Magnitude of SDH at Liquid Nitrogen Temperature*

Age	Addition	Arbitrary Units g = 1.94	g = 2.00	g = 2.01	$R_{1.94/2.00}$	$R_{1.94/2.01}$
0 Min.	None	46	7	19	6.6	2.4
125	"	22	8	10	2.8	2.2
0 Min.	Succinate, 5.8×10^{-2} M	57	12	26	4.8	2.2
110	"	37	17	15	2.2	2.5

*Experiments were performed under conditions as described in the legend of Fig. 1. The enzyme was aged in air at 0° C. The system in 0.2 ml contained 2.0 mg protein in 0.1 M phosphate buffer, pH 7.8.

which varied greatly with conditions. Statistical analysis which will be reported elsewhere verified this contention. In the presence of sodium dithionite, the signal at g = 2.00 completely disappeared, as did the yellow color, whereas the absorption at g = 1.94 and 2.01 reached maximal magnitude. Although sodium dithionite at room temperature also abolished the signal of SDH at g = 2.0029, some component of the reducing agent showed a strong signal at g = 2.0053 with a relatively narrow width of 31.3 gauss. However, the dithionite solution itself did not show a signal at liquid nitrogen temperature.

Beinert and Lee (1961) in their studies with submitochondrial particles have attributed the signals at g values 1.94 and 2.01 to a ferrous iron ligand configuration. But the evidence presented did not permit them to postulate whether these signals *** were due to one or two entities. In the case of soluble succinic dehydrogenase, the constant ratio now observed is evidence that these two signals are due to one functional group on the enzyme. We have been unable to detect the signal of ferric iron in these preparations; the reduction of ferric to ferrous iron must be accompanied by a change in ligand field, if the signal is in fact due to iron.

The signal observed with SDH at g = 2.00 may be equivalent to that reported by Commoner and Hollocher (1960) in heart homogenates and by Beinert and Lee (1961) in submitochondrial particles, and assigned by Beinert and Lee to flavin semiquinone. Commoner and Hollocher have shown that the signal is maximal at a fumarate succinate ratio of about 1.7. This was confirmed in the present study with purified SDH at room temperature; however, either at 25° or -196°, the signal was present in the absence of succinate or fumarate, and at -196°, all signals increased with increasing concentrations of succinate and decreased with increasing concentrations of fumarate as shown in Table I.

We studied the extreme instability of the enzyme with respect to its biological reaction with the cytochrome system. Table II summarizes the decrease of signal magnitude with age of the enzyme. It can be seen that the decay of

***The g_{11} = 1.94 and $g_{\perp}$ = 2.01 used by Beinert and Lee (1961) in this paper are equivalent to g = 1.94 and g = 2.01 respectively.

the "iron" signal was more rapid than that of the "semiquinone" signal. It has been shown previously (King, 1961) that the phenazine reaction of SDH is less sensitive than its reconstitutive capacity during aging of the enzyme. However, the rate of inactivation of the reconstitutive capacity was still higher than the decay of iron signals reported here. Another labile site may be involved in reconstitution but not directly participate in the electron transfer.

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