EVIDENCE FOR THE BINDING OF OXYGEN AND CARBON MONOXIDE BY SUCCINIC DEHYDROGENASE*

James B. Griffin and Thomas C. Hollocher

The Graduate Department of Biochemistry
Brandeis University, Waltham, Massachusetts 02154
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Summary. Electron spin resonance (ESR) studies suggest that soluble succinic dehydrogenase (SDH) from beef heart may bind O_2 in the presence of succinate at 30° and that the binding of CO may be recognized by its competitive action on O_2. The binding of CO can be observed optically in the presence of dithionite.

Experimental. Soluble SDH from beef heart was prepared after the acetone powder method of Singer et al. (1956) as modified by Griffin et al. (in press). The enzyme characteristics and the assays and analyses used were as described (Griffin and Hollocher, 1966; Griffin et al., in press). For ESR studies, gas phase exchange was accomplished in a small jet flow system using humidified gases of known composition. Small volumes of the enzyme preparation and required reactants were held in separate compartments in the apparatus so that mixing could be carried out at any time. When the gas phase was changed sample surfaces were exposed to a gas jet for 15 min. before initiating the next operation. Since the ESR sample capillary was a part of the apparatus, ESR observations could be made without exposing the sample to air. A gas washing train containing alkaline dithionite served to deoxygenate certain gas mixtures. ESR observations were made at 30° using SDH preparations 30 to 40 μM in flavin.

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Results and Disccussion. The ESR properties of soluble SDH near room temperature have been studied in some detail under aerobic conditions, and a tentative reaction scheme has been proposed (Hollocher and Commoner, 1961; Griffin and Hollocher, 1966; Griffin et al., in press). The development of maximum radical concentration aerobically depends on the simultaneous presence of succinate and fumarate. In the presence of added succinate alone, the concentration of radical is very low, as shown in Fig. 1. If, however, the system is made anaerobic prior to mixing SDH and succinate, a large radical yield results as seen in Fig. 1. The further addition of any amount of fumarate now decreases the radical concentration, contrary to the aerobic situation. Maximum radical concentration is 50 to 60% of the SDH concentration and represents a concentration double that obtainable under aerobic conditions in the presence of optimal concentrations of succinate and fumarate. Except for amplitude we have been unable to detect a change in ESR signal characteristics as a function of gas phase composition. The amplitude changes would appear therefore to reflect only changes in radical concentration.

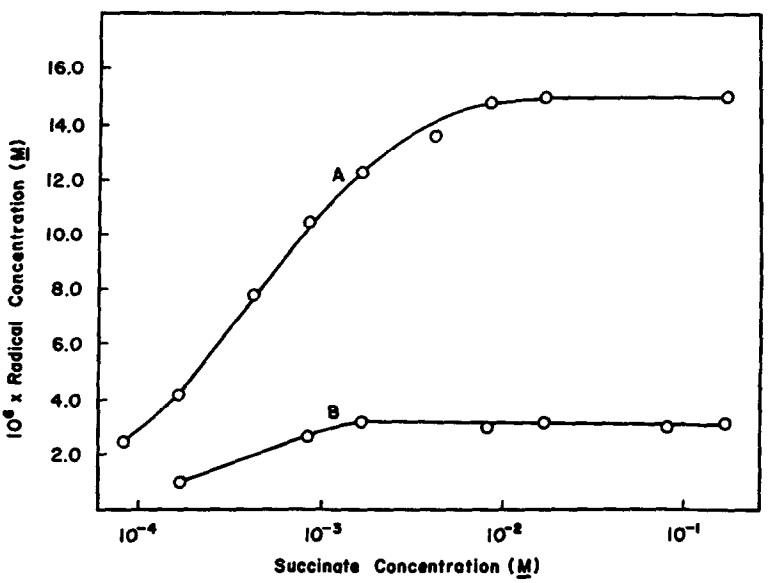

Fig. 1. Effect of succinate concentration on radical concentration. All samples contained 30 μM SDH flavin, 200 mM phosphate and succinate as indicated, all at pH 7.4. In semilog plots: A, under 1 atm N_2; B, under air.

When the O_2 concentration is varied prior to mixing, a graded effect of O_2 is observed which is illustrated in Fig. 2. Low concentrations of CO increase radical concentration in a manner suggesting a competition between CO and O_2. A half-effect value for O_2 of about 15 mm can be obtained from Fig. 2, but this value should not be considered a true dissociation constant since the value depends to some extent on the succinate concentration.

To a considerable extent the effect of O_2 on radical concentration is reversible and consistent with a binding phenomenon. In Table I we include data which show that a) CO at 1 atm enhances radical yield under otherwise anaerobic conditions, b) the high radical concentrations of anaerobic situations can be achieved to a large extent by the introduction of N_2 <u>after</u> mixing succinate and SDH under aerobic conditions, c) the aerobic situation can be achieved by introducting air <u>after</u> mixing succinate and SDH under anaerobic conditions, d) He and N_2 are equally effective in achieving anaerobic conditions.

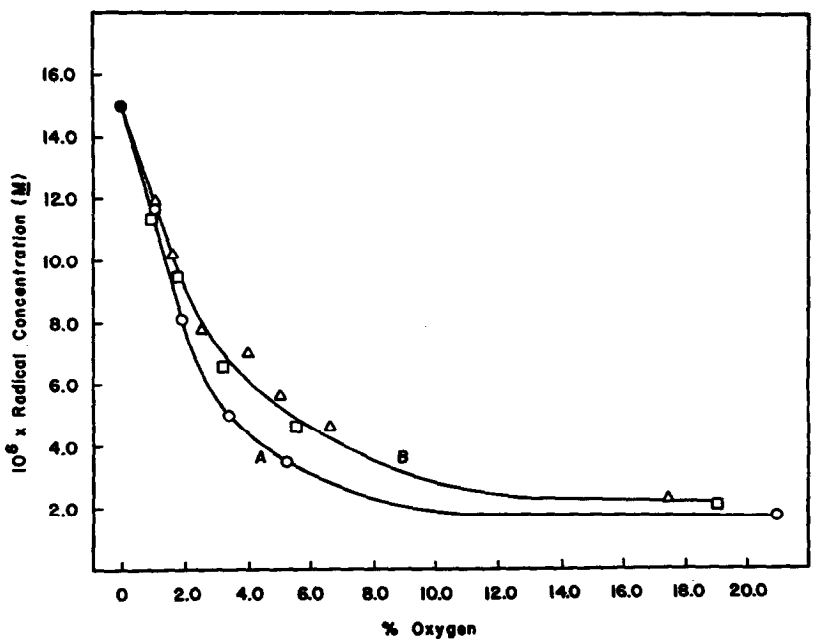

Fig. 2. Influence of O_2 and CO on radical concentration. All samples contained 30 μM SDH flavin, 17 mM succinate, and 200 mM phosphate, all at pH 7.4. A, under 1 atm N_2 + O_2 with the $\%O_2$ as indicated; B, under 1 atm of CO + N_2 + O_2 with the $\%O_2$ as indicated: Open squares, 9% CO; open triangles, 17% CO.

Table I

Effect of Gases on Radical Concentration in SDH

After mixing all samples contained 30 to 40 μM SDH flavin, 17 mM succinate, and 200 mM phosphate at pH 7.4.

Gas Phase (1 atm)			Relative Radical Conc. (%)
Initial	At mixing	For ESR	
N_2	N_2	N_2	100
He	He	He	101
CO	CO	CO	140
Air	Air	Air	20
O_2	O_2	O_2	20
N_2	N_2	Air	30
N_2	Air	Air	20
CO	Air	Air	20
Air	Air	N_2	70
Air	Air	CO	70

Irreversible effects attributable to the autoxidation of succinate would appear to be minimal. We have been unable to detect autoxidation of succinate by high concentrations of SDH in the temperature range 23° to 30° using three methods. The methods and their estimated sensitivities are manometric, 0.3 moles O_2/min/mole SDH flavin; vibrating oxygen electrode, 0.025 moles O_2/min/mole SDH flavin; ESR, 0.02 moles O_2/min/mole SDH flavin. The latter method depends on the appearance of fumarate as a consequence of autoxidation, and the known effect of the fumarate/succinate mole ratio on ESR signal intensity under aerobic conditions serves as calibration. The negative results simply confirm the generally held view that SDH is not autoxidizable.

The ESR method gives no information about the interaction of O_2 or CO with SDH alone since succinate is required before radicals can be detected. It is possible, therefore, that succinate may enhance or create the O_2-binding capacity of SDH.

The matter of CO binding by SDH was anticipated by King's (1965) observation of a spectral effect of CO on a nonheme iron peptide obtained from SDH following proteolytic digestion. When the peptide was reduced by dithionite, CO caused a spectral perturbation from below 400 mμ to above 500 mμ, with maximum effects in the region from 400 to 450 mμ. We find a related effect of CO on the spectrum of our SDH preparations in the presence of dithionite, as shown in Fig. 3. The large CO absorption band

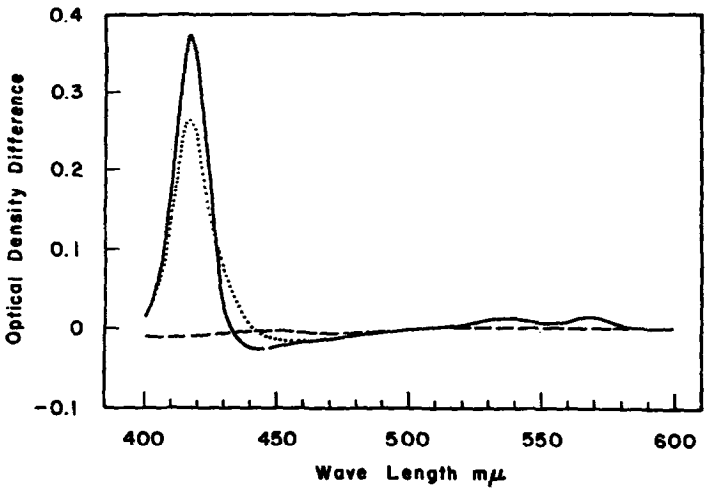

Fig. 3. Difference spectra showing a CO band in SDH preparations at 417-418 mμ in the presence of dithionite. ---, SDH minus SDH; _____, under 1 atm CO minus under 1 atm N_2, both containing a few grains of dithionite; ....., above curve corrected for a contribution due to hemoglobin (or myoglobin). The SDH preparation used was purified only by ammonium sulfate fractionation and contained 1.2 μM heme as hemoglobin (or myoglobin), as determined from cyanide difference spectra in the presence of 0.1 mM ferricyanide. MetHbCN minus MetHb, $\Delta\epsilon$(mM heme, 423 mμ minus 404 mμ) = 142. The correction due to the HbCO minus Hb difference spectrum was based on hemoglobin standards of known concentration and employed a $\Delta\epsilon$(mM heme, 419 mμ minus 433 mμ) of 150. The samples contained 0.1 M phosphate buffer, pH 7.6. The SDH preparation was about 30 μM in flavin and 1 cm cuvettes were used. For the CO band of SDH a $\Delta\epsilon$(mM flavin, 418 mμ minus 455 mμ) of about 9 is estimated.

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is not observed with soluble SDH alone as prepared by the above method, with SDH in the presence of succinate, or with SDH which has been reoxidized after exposure to dithionite (unless CO is present during the autoxidation of excess dithionite). As yet we have been unable to observe effects of O_2 or CO on the catalysis of succinate oxidation in systems where ferricyanide or phenazine methosulfate and dichlorophenolindophenol (in sequence) serve as terminal oxidants.

The present ESR data provide no information concerning the sites of interaction of O_2 and CO with SDH. By analogy with hemoproteins, hydrogenase, and hemerythrin, we would suppose that binding occurs at iron atoms which have some ferrous character. Low temperature ESR observations support the view that succinate

can reduce at least a portion of the iron of SDH (Beinert, 1966). In this connection it is interesting to suggest that the unusual effect of O_2 on the low temperature ESR spectra of SDH, reported by Veeger et al. (1963) and Dervartanian (1965), may be due to O_2 binding. Oxygen was observed to decrease the semiquinone signal at g 2.00 in the presence of succinate and to increase the nonheme-iron signals at g 2.01 and 1.94. The latter effect is unexpected if O_2 were serving as an oxidant of SDH.

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