Regulation of Succinate Dehydrogenase in *Escherichia coli*

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SUMMARY

Washed suspensions of *Escherichia coli* oxidized succinate when previously grown on succinate but not on glucose. A slower rate of oxidation occurred with bacteria grown with peptone as carbon source. These differences were due to alterations in the level of succinate dehydrogenase activity. Glucose repressed the biosynthesis of the enzyme, whereas succinate acted as specific inducer and did not increase the activity of fumarate hydratase, malate dehydrogenase or NADH dehydrogenase. Aerobic growth also increased the levels of membrane-bound succinate dehydrogenase. Induction of succinate dehydrogenase by added succinate followed the expected kinetics. Addition of glucose caused a decline in the rate of biosynthesis of succinate dehydrogenase. Succinate dehydrogenase appears to play an important respiratory role since amytal (an NADH-oxidase inhibitor) inhibited growth only slightly when succinate was used as carbon source as compared to the strong inhibition of growth when glucose was used as carbon source.

INTRODUCTION

Succinate dehydrogenase is the only membrane-bound enzyme of the tricarboxylic acid cycle in *Escherichia coli* (Marr, 1960) and, therefore, it may participate in respiration besides functioning in the tricarboxylic acid cycle. Changes in the environment may consequently affect both respiratory activity and the functioning of the tricarboxylic acid cycle. Influence of growth conditions on the biosynthesis and activity of membrane-bound dehydrogenases and cytochromes of bacteria is well documented (Gray, Wimpenny & Mossman, 1966; Cavari, Avi-Dor & Gressowicz, 1968; Ruíz-Herrera & De Moss, 1969). Preliminary studies (Ruíz-Herrera, 1968) have suggested that the carbon growth substrate was important in governing the synthesis of succinate dehydrogenase in *E. coli*. This study presents further data on the mechanisms which control the biosynthesis of succinate dehydrogenase in this bacterium.

METHODS

Organism and growth conditions. *Escherichia coli* HfrH, obtained from J. A. de Moss, University of California, San Diego, U.S.A., was maintained on nutrient agar (Difco) and grown on the medium described by Sypherd & Strauss (1963) with 5 µg of thiamine/ml and either 1 % glucose or 0.5 % succinate. This medium is referred to as 'synthetic medium'; for 'complex medium', nutrient broth (Difco) at 0.4 % was added. Bacteria were grown at 37 °C for 4 to 5 h, the medium being sparged with about 2 volumes of sterile air/volume of culture/min. For anaerobic conditions, cultures were sparged with a mixture of 95 % N₂ + 5 % CO₂. Bacterial density was measured by means of a Klett photocolormeter using a green filter and the protein content was calculated from the turbidity with an appropriate calibration curve.

Preparation of bacteria-free extracts and bacterial envelopes. Bacteria were centrifuged, washed with 0.05 M-phosphate buffer, pH 7.3, and broken with a 10 kHz Branson Sonifier (Heat Systems Ultrasonics, Plainview, New York, U.S.A.) for three successive periods of 15 s each. The extract was centrifuged at 400g for 15 min and the supernatant used as crude extract. Bacterial envelopes were isolated by resuspending the bacteria in 0.5 ml of 0.05 M-tris-HCl buffer, pH 8.3, containing 20 % sucrose and 2 mg lysozyme (Sigma Chemical Co. Inc., St Louis, Missouri, U.S.A.)/ml and frozen in an acetone-solid CO₂ mixture. After thawing at 37 °C, the treatment was repeated four times. Five ml of 0.05 M-phosphate buffer containing 10 µg DNase (Sigma Chemical Co.)/ml were added and rapidly mixed until the viscosity decreased. The crude extract was centrifuged at 50 000g for 10 min, the supernatant removed and the residue resuspended in 1 ml of phosphate buffer and centrifuged at 500g in a linear sucrose gradient (50 to 20 %) for 20 min. Bacterial envelopes appeared as an opalescent band within the gradient.

Determination of respiratory activity. Oxygen uptake was measured with a Clark oxygen electrode (Yellow Spring Instrument Co. Inc., Ohio, U.S.A.), attached to a Perspex cylindrical chamber with a capacity of 3.2 ml.

Measurement of succinate uptake. Samples (5 ml) of bacterial suspensions in 0.05 M-phosphate buffer, pH 7.3, at a turbidity of 360 Klett units (= 1.4 mg dry wt bacteria/ml) were incubated with 0.2 ml 0.05 M-[1,4-¹⁴C₂]succinate (sp.act. 0.01 µCi/µmol) (Calbiochem Inc., La Jolla, California, U.S.A.). At intervals, 1 ml samples were removed and mixed with 1 ml 0.1 M-KCN (at 0 °C) and filtered through membrane-filters (0.47 µm pore diameter, Millipore Corporation, Bedford, Massachusetts, U.S.A.). The bacteria were washed with 5 ml of ice-cold 0.1 M-KCN. Radioactivity of the cells was measured with a Geiger counter (Phillips, Model 4035) which had an absolute efficiency of 2.5 % for ¹⁴C. No correction for self-absorption was made.

Enzymatic activities. Succinate dehydrogenase was measured by the method of Ells (1959). Rate of dichloro-indophenol reduction was measured with a Maroc V (Jobin et Ivon, Arcueil, France) spectrophotometer coupled to a Photo-Volt Model 43 recorder. Activity was expressed as $\Delta E_{600\text{ nm}}/\text{min/mg}$ of protein. Fumarate hydratase was measured by the method of Massey (1955), and activity expressed as $\Delta E_{300\text{ nm}}/\text{min/mg}$ of protein. Malate dehydrogenase was measured as described in the brochure *Enzymes, Enzyme Reagents* from Worthington Biochemical Corp., Freehold, New Jersey, U.S.A. Activity was expressed as $\Delta E_{340\text{ nm}}/\text{min/mg}$ protein. NADH dehydrogenase was measured as follows: 0.2 ml of 0.0015 M-NADH was mixed with 2.7 ml of 0.05 M-phosphate buffer, pH 7.3, and 0.2 ml of bacterial extract. Activity was measured as described for succinate dehydrogenase and expressed as $\Delta E_{340\text{ nm}}/\text{min/mg}$ protein.

Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Succinate was not as good a carbon source as glucose for the growth of *Escherichia coli*. When both substrates were added to a synthetic medium, diauxic growth occurred with glucose being used first. The effect of glucose was attributed to alterations in the respiratory activity as glucose-grown bacteria did not oxidize succinate (Table 1). Differences in respiratory activity between glucose- and succinate-grown bacteria correlated with the activity of succinate dehydrogenase in extracts from the two types of bacteria (Table 1). However, permeability to [1,4-¹⁴C₂]succinate was not greatly affected by the conditions of growth (Fig. 1).

Fig. 1. Uptake of $[1,4-^{14}\text{C}]$ succinate into *Escherichia coli*. Bacteria were grown for 4 h in complex medium alone or with succinate or glucose added. Uptake of $[1,4-^{14}\text{C}]$ succinate was followed as described in Methods. ○, Succinate-grown bacteria; ●, glucose-grown bacteria; ×, bacteria grown in complex medium alone.

Table 1. Effect of carbon source on respiratory and succinate dehydrogenase activities

Medium	Additional carbon source	Respiratory activity Substrate		Specific activity of succinate dehydrogenase ($\Delta E_{800}/\text{min}/\text{mg}$ protein)
		Glucose ($\mu\text{l O}_2/\text{min}/\text{mg}$ protein)	Succinate ($\mu\text{l O}_2/\text{min}/\text{mg}$ protein)	
Synthetic	Glucose	6.9	0	0.17
	Succinate	3.6	4.9	1.32
Complex	None	—	2.1	0.12
	Glucose	—	0	0.04
	Succinate	—	6.3	0.39

Isolated bacterial envelopes readily oxidized succinate and NADH, but not glucose. NADH oxidation by envelopes from glucose- and succinate-grown bacteria was about the same but succinate was oxidized more rapidly by envelopes from succinate-grown bacteria. These differences were related to the activity of the corresponding enzymes (see Table 2).

The effect of succinate as inducer of succinate dehydrogenase was specific since its addition to complex medium significantly increased the synthesis only of succinate dehydrogenase, whereas fumarate hydratase and malate dehydrogenase were not affected (Table 3). Glucose did not repress the latter two enzymes as much as it did succinate dehydrogenase (Table 3).

The absence of oxygen during growth also affected the respiratory activity and the levels of succinate dehydrogenase. Bacteria grew poorly with succinate as carbon source under anaerobic conditions and had 25 % of the respiratory activity with succinate and only 8 %

Table 2. *Enzymatic and respiratory activities in envelopes isolated from Escherichia coli grown aerobically in complex medium*

Additional carbon source	Respiratory activity. Substrate		Specific activities of	
	Succinate ($\mu\text{l O}_2/\text{min}/\text{mg protein}$)	NADH	Succinate dehydrogenase ($\Delta E_{400}/\text{min}/\text{mg protein}$)	NADH dehydrogenase ($\Delta E_{340}/\text{min}/\text{mg protein}$)
None	—	—	0.89	3.8
Glucose	0.59	5.15	0.44	5.2
Succinate	6.59	4.11	4.44	3.8

Table 3. *Effect of culture condition on the synthesis of several enzymes from the tricarboxylic acid cycle from Escherichia coli grown aerobically in complex medium*

Additional carbon source	Enzyme activity		
	Succinate dehydrogenase*	Fumarate hydratase†	Malate dehydrogenase‡
None	0.270	0.0054	0.350
Glucose	0.022	0.0056	0.161
Succinate	0.850	0.0077	0.360

* $\Delta E_{400}/\text{min}/\text{mg protein}$.† $\Delta E_{300}/\text{min}/\text{mg protein}$.‡ $\Delta E_{340}/\text{min}/\text{mg protein}$.

of succinate dehydrogenase activity as compared with bacteria grown aerobically. The amount of succinate dehydrogenase present in glucose-grown cells was independent of the oxygen concentration.

Kinetics of succinate dehydrogenase induction were measured under conditions where succinate was not the limiting factor for growth (Fig. 2). After succinate was added to the growth medium, the induction of the enzyme followed the classical kinetics described by Jacob & Monod (1961) for an inducible system. When chloramphenicol (50 $\mu\text{g}/\text{ml}$) was added simultaneously with succinate, no increase in activity of succinate dehydrogenase was obtained.

In a further experiment repression by glucose of the enzyme system in bacteria growing on succinate was followed (Fig. 3). Synthesis of succinate dehydrogenase stopped immediately after glucose was added to the medium and existing enzyme decayed rapidly indicating that either it has a rapid turnover rate or that glucose favours its degradation or inactivation.

When glucose was removed from succinate-containing medium, enzyme synthesis was immediately restored following similar kinetics to that shown in Fig. 2.

The role of succinate dehydrogenase in respiration of the bacterium was investigated by observing the effect of amytal on growth. Amytal inhibits NADH dehydrogenase of mitochondria (Chance & Hollunger, 1963) and also NADH oxidase from *Agrobacterium tumefaciens* (Kurup, Vaidyanathan & Ramasarma, 1966). With an envelope preparation from *Escherichia coli* 0.6 mM-amytal inhibited NADH oxidation by 40 % but had no effect on succinate oxidation. If amytal inhibited NADH oxidation, but not succinate oxidation, addition of amytal to a medium with substrates which were oxidized via NAD would bring about

Fig. 2. Induction of succinate dehydrogenase in *Escherichia coli*. *Escherichia coli* was grown in aerated complex medium for 130 min and 0.5% succinate then added. Samples were removed, washed in buffer containing 50 μ g chloramphenicol/ml and sonicated. Specific activity of succinate dehydrogenase, ○, is expressed as $E_{600}/\text{min}/\text{mg}$ protein. Total activity, ●, is for 1 ml culture medium.

Fig. 3. Repression of succinate dehydrogenase by glucose in *Escherichia coli*. Bacteria were grown in complex medium for 3 h and 0.75% glucose was added. At intervals samples were removed and treated as described for Fig. 2. Specific activity, ○, and total activity, ●, are expressed as in Fig. 2.

a stronger inhibition than when it was added to a medium where the respiratory substrate was succinate. Thus 0.1 mM-amytal inhibited growth of *E. coli* in complex medium by 30 % when glucose was present but only by 10 % when succinate was added instead of glucose. When 1 mM-amytal was used, growth inhibition was 60 % and 20 % respectively.

DISCUSSION

The formation of succinate dehydrogenase in *Escherichia coli* is controlled by the environmental conditions. Its repression by glucose is another example of an enzyme subject to catabolite repression (Magasanik, 1961). Glucose had little effect on the synthesis of malate dehydrogenase and the permeability system for succinate. Glucose does repress the synthesis of succinate dehydrogenase in *Haemophilus parainfluenzae* (White, 1967) and *Staphylococcus aureus* (Collins & Lascelles, 1962). With *E. coli* grown in complex medium, the formation of enzymes of the tricarboxylic acid cycle is also repressed by glucose (Gray *et al.* 1966). We found a more specific effect of glucose since fumarate hydratase was not repressed by the carbohydrate.

The induction of succinate dehydrogenase in *Escherichia coli* HfrH by succinate is similar to that reported by Cavari *et al.* (1968) who found that the activity of succinate dehydrogenase increased about sixfold when *E. coli* was grown in a succinate-containing medium as compared to the activity shown by bacteria grown in a medium containing fumarate or mannitol.

Our data on the levels of succinate dehydrogenase in aerobically and anaerobically grown cells agree with those reported by Gray *et al.* (1966), Hino & Maeda (1966), and Cavari *et al.* (1968). Location within the membrane confers special characteristics to succinate dehydrogenase, as has been discussed by Cerletti, Gioveno, Testolin & Binotti (1968); of special interest is that succinate dehydrogenase may have a respiratory role according to our results.

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