REPRESSION OF OXIDATIVE PHOSPHORYLATION IN ESCHERICHIA COLI B BY GROWTH IN GLUCOSE AND OTHER CARBOHYDRATES

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Summary

A simplified method for the assay of oxidative phosphorylation in intact bacteria is described. Using this method it is shown that the efficiency of oxidative phosphorylation in Escherichia coli B grown in glucose-containing media is less than 10% of that of organisms grown in media containing non-carbohydrate substrates. Growth in media containing galactose, lactose or mannitol yields cells capable of only half the efficiency of oxidative phosphorylation of fully competent cells. The incorporation of casamino acids into the growth medium has no effect on glucose repression of oxidative phosphorylation. Stimulation of respiration by the uncoupler 2,4-dibromophenol occurs only in organisms in which all three sites of oxidative phosphorylation are functional.

Introduction

Escherichia coli B, grown in tryptic soy broth and harvested in the stationary period of growth, is capable of oxidative phosphorylation with an apparent efficiency of 3.5 ± 0.3 moles P_i esterified per mole of NADH oxidized[1]. Bacteria harvested during the logarithmic growth period show considerably lower efficiency of phosphorylation, however, apparently due to the presence of glucose. Using a method of assay of oxidative phosphorylation in intact cells based on that previously described it can be shown that cells growing aerobically in a variety of media carry out oxidative phosphorylation at efficiencies which are a function of the composition of the growth medium. The results suggest that part of the enzymatic or structural apparatus of oxidative phosphorylation is subject to catabolite repression.

Materials and Methods

Escherichia coli B was maintained as described[1].

The media employed were Fisher Tryptic Soy Broth and, to test the effects of growth in the presence of various substrates, a solution of the following composition: $MgSO_4 \cdot 7H_2O$ - 0.8 g/l; NH_4Cl - 0.5 g/l; NaCl - 0.3 g/l; trace metals[2] - 10 ml/l; potassium phosphate - 30 mM, pH 7.0; Difco Casamino Acids - 5 g/l; and substrate - 15 mM.

E. coli B was cultivated in the medium to be used for a period of 12 hr before inoculation (5%, v/v) into 700 ml of medium in 2.8 l Fernbach flasks. Cultures were shaken at 300 rpm on a New Brunswick platform shaker at 37°. When examining cells grown in tryptic soy broth flasks were removed at the times stated, the contents were immediately chilled in ice, and then harvested within 10 min. When the effect of various substrates was tested flasks were removed when the optical density at 650 nm reached 1.0 ± 0.1 (2.5 to 6 hr after inoculation) and the contents immediately harvested by centrifuging in the cold at 12,000 x g for 10 min. Cells were washed twice by resuspending in cold Tris-Cl buffer, 0.1 M, pH 7.5, and were finally suspended in the same buffer at a concentration of about 15 mg/ml (dry weight) and kept in ice until used.

The respiratory activity of bacterial suspensions was measured at 23-25° with a Clark oxygen electrode in a vessel of 3.5 ml volume. To initiate respiration 3 mM glucose was added to a washed cell suspension (0.6 - 0.8 mg/ml) in 0.1 M Tris-Cl buffer, pH 7.5

Glucose in the supernatant portion of the culture fluid was assayed by the anthrone procedure[3].

To assay oxidative phosphorylation twelve 1 ml suspensions of bacteria were incubated at 30° for 7 min under a stream of He[1]. At zero time 20 µl of Tris-phosphate^{32}, pH 7.0, 0.5 M, specific activity 500-1000 cpm/mµmole, was added to each of 4 samples (Set 1). To each of 4 other samples (Set 2) was added 5 µl of 0.1 M 2,4-dibromophenol in methanol (DBP) 30 sec before addition of P_i^{32}. The 4 samples remaining (Set 3) received 20 µl of 0.5 M Tris-P_i, pH 7.0. The P/2e$^-$ value finally expressed is the amount of P_i^{32} esterified after

5 sec of exposure to oxygen (Set 1) minus the amount incorporated in the presence of the uncoupler (Set 2) divided by the amount of NADH oxidized (Set 3). 15 min of incubation under He assured isotopic equilibration of P_i^{32} between the intra- and extracellular spaces. Accordingly, at 15 min after P_i^{32} addition 1 ml of aerobic Tris-Cl buffer, 0.1 M, pH 7.5, was rapidly injected into two samples of Set 1 and two samples of Set 2 followed 5 sec later by 1 ml of a solution of 1.8 M $HClO_4$ and 1.2 M Na_2SO_4[4]. To each of two samples of Set 3 was added 1 ml of aerobic Tris-Cl buffer followed 5 sec later by 1 ml of a solution of 1.4 M KOH in 85% (v/v) ethanol[1]. The remaining samples of each set received 1 ml of the terminating reagent 5 - 10 sec before the addition of aerobic buffer. The acid-treated samples were centrifuged in the cold to remove denatured material and 1 ml of the supernatant portion of each was removed for the measurement of total and organic phosphate by the method of Hagihara and Lardy[4]. An Ansitron Mk II scintillation counter was used.

NADH was determined in heated, neutralized portions of the samples of Set 3 as previously described [1].

Results

Table 1 shows the results of an experiment in which the time course of growth, glucose utilization and phosphorylation efficiency was followed. E. coli B growing in tryptic soy broth is incapable of full oxidative phosphorylation although within 90 min after the exhaustion of glucose the ability to carry out full oxidative phosphorylation is gained. That the acquisition of oxidative phosphorylation is dependent upon growth is demonstrated by the observation that chloramphenicol, added at the time glucose is exhausted, prevents both growth and the development of oxidative phosphorylation. The molar growth yield of such a culture, at the time of glucose disappearance, is 60 - 70 g dry weight per mole of glucose utilized.

Other compounds were tested for the ability to affect the efficiency of oxidative phosphorylation of cells grown in their presence, and the results

Table 1. Efficiency of oxidative phosphorylation in E. coli B during growth in tryptic soy broth.

Time	Cell[a] density	Residual glucose	ΔP_i[b]	ΔNADH[b]	Apparent P/2e$^-$
0 hr	0.07	14.2 mM	-	-	-
2.0	0.62	9.2	0.47	1.43	0.33
3.5	1.92	0.6	0.30	0.82	0.36
5.2	2.48	0	3.69	1.13	3.3
5.8	2.70	0	3.48	0.81	3.9
6.3[c]	1.84	-	0.12	0.53	0.23

[a]Optical density at 650 nm.

[b]μmoles/g dry weight.

[c]30 μg/ml chloramphenicol added at 3.6 hr.

are given in Table 2. Three classes of compounds were separable. Organisms grown in media containing Class A compounds are capable of full oxidative phosphorylation and DBP increases the rate of oxygen consumption during glucose oxidation some 1.7 - fold. The mean P/2e$^-$ value found is less than that previously reported because of dilution of P_i^{32} (some 5 - 10%) by adventitious P_i. Organisms grown in a medium containing glucose (Class C) do not carry out significant oxidative phosphorylation, even when the medium also contains a Class A compound, and their rate of oxygen uptake during glucose oxidation is not much increased by DBP. Cells grown in media containing Class B compounds are capable of oxidative phosphorylation intermediate between that of cells grown in media containing Class A compounds and those containing glucose, but their rate of oxygen consumption during glucose oxidation is changed only little by the addition of DBP. The amounts of P_i esterified during the 15 min anaerobic preincubation period differ according to the class of substrate employed for growth in a manner perhaps related to

Table 2. Oxidative phosphorylation in E. coli B grown in the presence of various substrates.

Substrate	ΔP_i[a]	ΔNADH[a]	Apparent P/2e$^-$	Anaerobic[b] ΔP_i	Glucose[c] oxidation	RCR[d]
Class A						
None	4.63	1.52	3.1	4.05	-	-
Formate	3.52	1.06	3.3	7.05	40	1.45
Acetate	6.24	1.61	3.8	6.24	48	1.80
Lactate	6.07	1.83	3.3	9.14	67	1.81
Glycerol	3.60	1.14	3.2	8.83	62	1.47
Succinate	4.80	1.70	2.8	8.21	61	1.87
Malate	5.36	1.92	2.8	8.73	61	1.88
Glutamate	4.15	1.08	3.8	7.14	34	1.72
Ribose	4.60	1.18	3.8	10.63	33	1.51
Mean (12)[e]			3.3	7.9		1.7
S.D.			0.4	1.0		0.2
Class B						
Lactose	3.16	1.91	1.7	9.00	58	0.93
Mannitol	1.98	1.00	2.0	5.76	38	1.00
Galactose	2.25	1.15	2.0	7.14	52	1.14
Mean (6)			1.7	7.1		1.0
S.D.			0.3	1.1		0.1
Class C						
Glucose	0.17	1.83	0.10	2.36	88	1.15
Glucose + glycerol	0.31	2.08	0.15	1.74	74	0.92
Glucose + lactate	0.58	3.03	0.20	2.10	65	1.19
Mean (6)			0.2	2.0		1.1
S.D.			0.1	0.3		0.1

[a] µmoles/g dry weight.

[b] P_i esterified during 15 min anaerobic preincubation (µmoles/g dry weight).

[c] µM O_2/min/mg.

[d] Respiratory Control Ratio: ratio of steady state glucose oxidation rate in the presence of 140 µM DBP to that in the absence of DBP.

[e] Number of samples.

the ability to carry out oxidative phosphorylation. All preparations are capable of glucose oxidation as well being able to oxidize the compound tested.

Variation of the concentration of casamino acids (zero to 10 g/l) had no effect on the ability of glucose to repress oxidative phosphorylation, although it is possible that repression by Class B compounds could be affected by the presence or absence of added carbon sources.

Discussion

Growth in glucose appears to repress the synthesis of components common to all three sites of oxidative phosphorylation. Derepression of synthesis is growth dependent as indicated by the response to chloramphenicol, but it is not known if derepression is brought about by the synthesis of new proteins, membranes, a combination of the two, or some other component. Massive synthesis of new cytochromes and flavoproteins is not involved (W.P. Hempfling, in preparation) although changes of the kinds and amounts of quinones present have not been excluded. Class B compounds bring about partial repression of oxidative phosphorylation, but it is not clear whether a fraction of the population is totally unable to carry out oxidative phosphorylation with the remainder fully competent or if one or two sites of phosphorylation are repressed uniformly throughout the population. Reports have appeared of the absence of a single phosphorylation site (Site I) in yeast mitochondria[5,6], and glucose has been shown to repress the synthesis of yeast mitochondria[7].

Cavari, et al[8] have provided evidence that E. coli, grown anaerobically in a mannitol-containing medium, is capable of less uncoupler-sensitive aerobic P_i esterification than is the same organism grown aerobically. Their methods did not permit the calculation of changes of phosphorylation efficiency, but they did note an increased effect of the uncoupler m-Cl-CCP in respiration following a shift from anaerobic to aerobic conditions. Like DBP, m-Cl-CCP stimulates oxygen consumption during glucose oxidation by E. coli B[9].

Hernandez and Johnson[10] found that the aerobic molar growth yield of glucose-grown E. coli was considerably lower than predicted if it was assumed that oxidative phosphorylation operated. However, Hadjipetrou, et al[11] reported that the aerobic molar growth yield on glucose of Aerobacter aerogenes was

that expected from the operation of three sites of oxidative phosphorylation. Such inconsistencies might be resolved by an investigation of the possible repressive effects on oxidative phosphorylation of the substrates used.

The results reported here extend the repressive properties of glucose and related compounds. To what extent repression of oxidative phosphorylation affects repression of other systems is as yet undetermined, but it is possible that some effects of glucose on enzyme synthesis in growing microorganisms may be due to a general deficiency in the ability to conserve energy through terminal electron transport.

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