RESPIRATORY CONTROL IN *ESCHERICHIA COLI*

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1. Introduction

In mitochondria the rate of respiration in the absence of ATP synthesis is usually considerably lower than maximal. Addition of ADP and phosphate stimulate respiration, a phenomenon termed 'acceptor control' of respiration. After conversion of ADP and P_i to ATP, respiration again slows. The ratio of the rates of respiration in the presence (state 4) and absence (state 3) of ADP and P_i is called the 'respiratory control index' or RCI. Uncouplers of oxidative phosphorylation also release respiratory control. The common factor in loss of respiratory control is a decrease in the steady-state level of the protonmotive force. Thus, proton uptake during ATP synthesis or proton–solute cotransport, or use of the membrane potential for similar processes allows for an increase in the rate of proton extrusion by the electron-transport chain, that is, release of respiratory control.

While respiring cells of *Escherichia coli* do not exhibit respiratory control, respiration in resting cells is stimulated by addition of uncoupler [1]. Although uptake of solutes via proton–solute symports would be expected to decrease the steady-state protonmotive force, only marginal stimulation of respiration under such conditions was observed [1]. Moreover, in [1] there was apparently no correlation between the proton permeability of strains defective in the proton-translocating ATPase (F_0F_1) and loss of respiratory control.

Here, we confirm and extend the observations in [1] to show that substrates of the lactose permease release respiratory control. In addition, a correlation was observed between the proton permeability of proton-translocating ATPase mutants and loss of respiratory control.

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2. Materials and methods

2.1. *Bacterial strains and cultivation*

Escherichia coli ML308-225 [2] is constitutive for lactose transport but lacks β-galactosidase. ML308-225 was grown at 37°C in minimal medium [3] supplemented with 1% bactopeptone. *E. coli* strain 7 is inducible for lactose transport. Strain NR70, derived from strain 7, lacks the F_1 portion of the F_0F_1; since it retains a functional F_0 or proton channel, it is proton permeable [4,5]. Strain NR71 is a proton-impermeable derivative of strain NR70 [6]. Strains 7, NR70 and NR71 were grown in minimal medium [3] supplemented with 54 mM glycerol. Cells were harvested in the later exponential phase of growth, washed twice with 0.12 M potassium phosphate buffer (pH 7.1) and resuspended in the same buffer to ~1 mg cellular protein/ml.

2.2. *Assays*

Oxygen consumption was measured polarographically in 2 ml cell suspension in 0.12 M potassium phosphate buffer (pH 7.1) using a Yellow Springs International Clark oxygen electrode. Cells were first starved by the method in [1]. The time necessary to reduce endogenous respiration rates varied with the strain; e.g., strains 7 and NR71 required longer periods of starvation than did NR70 to attain the same low level of endogenous respiration. Protein concentrations were determined by a modification of the method in [7].

2.3. *Reagents*

Thiomethyl-β-D-galactoside (TMG), lactose and melibiose were obtained from Sigma Chemical Co. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was purchased from Boehringer Mannheim Co. All other chemicals were obtained from commercial sources and were of reagent grade.

3. Results

Cultures of ML308-225 starved by the method in [1] exhibited low levels of endogenous respiration. Addition of the uncoupler FCCP stimulated oxygen consumption by a factor of 3 or greater (fig.1A). If the cells were metabolizing a carbon source such as lactate, FCCP did not stimulate (fig.1B).

When lactose, TMG or melibiose, all substrates of the lactose-transport system, were added, an increase in respiration was observed (fig.2). It is probable that the increase in respiration was due to sugar transport and not metabolism for two reasons:

(i) Since ML308-225 lacks β-galactosidase, the stimulation by lactose (fig.2A) is probably not due to lactose metabolism.

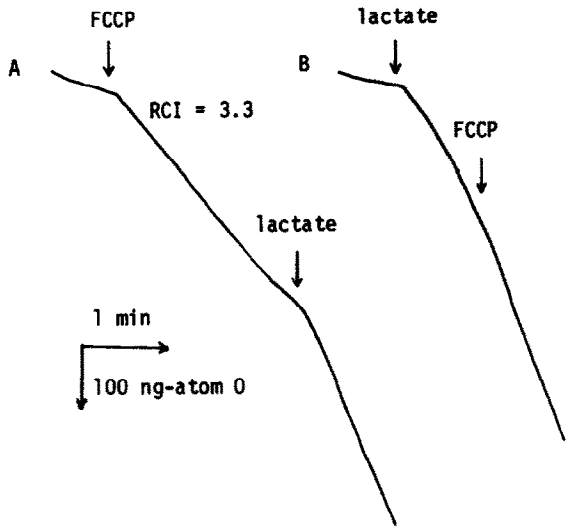

Fig.1. Stimulation of respiration in starved cells of strain ML308-225 by FCCP. Cells were starved for 10 min and respiration measured as described in section 2. (A) FCCP was added as an ethanol solution to a final concentration of 5 μM at the indicated time. (B) At the indicated time D-lactate and FCCP were added at 10 mM and 5 μM final conc., respectively.

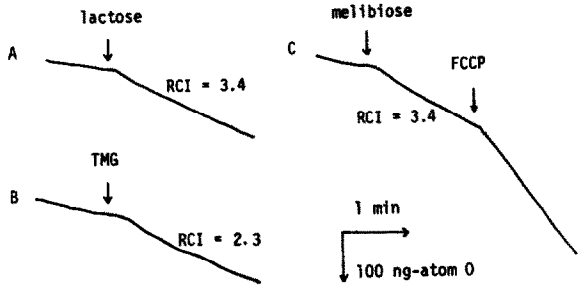

Fig.2. Effect of sugar–H^+ cotransport on the respiration rate. Cells were starved for 10 min and respiration measured as described in section 2. Lactose, TMG and melibiose were each added to 10 mM final conc. at the indicated times.

(ii) It is unlikely that the stimulation was due to small amounts of glucose or galactose contamination since uninduced cultures of *E. coli* strain 7 did not show an increase in respiration under the same conditions (not shown).

FCCP caused a further increase in respiration (fig.2C), suggesting that sugar transport was not sufficient to effect a complete transition from state 3 to state 4 respiration.

E. coli strain 7 exhibited a much greater release of respiratory control than did ML308-225, with RCI values of 10–20 compared to 3–6 for the ML strain. Strain NR70, which lacks the F_1 portion of the H^+-translocating ATPase, is proton permeable [4]. In this strain the RCI values were generally 3–4-fold less than in the parent, strain 7 (table 1). Strain NR71, a proton-impermeable derivative of NR70 [6], had RCI values comparable to that of the wild-type (table 1). It should be pointed out that, since each strain had to be starved for different

Table 1
Respiratory control in strains with mutations in the H^+-translocating ATPase

Strain	Respiratory rate (ng atom O . min^{-1} . mg $protein^{-1}$)		RCI[b]
	Endogenous[a]	+ 5 μM FCCP	
7	32	384	12.0
NR70	25	79	3.2
NR71	20	193	9.7

[a] Starvation times were 30 min for strains 7 and NR71, 15 min for strain NR70

[b] RCI = (respiratory rate + FCCP)/(endogenous rate of respiration)

times, the initial rate of endogenous respiration is irrelevant; the important factor is the RCI.

4. Discussion

Unlike mitochondria, growing cells of *E. coli* do not exhibit respiratory control. Non-growing cells were reported [1] to have a form of respiratory control characterized by an increase in the endogenous rate of oxygen consumption produced by addition of proton conductors. This suggests that the proton conductance of the membrane is the limiting factor for respiration under resting conditions. In growing cells the rate of proton translocation by the respiratory chain is in near equilibrium with the rate of proton uptake or the rate of utilization of the membrane potential by all of the energy-consuming pathways of the cell; thus, respiration is not limited by the protonmotive force, and respiratory control does not occur.

Active transport systems are major consumers of the protonmotive force [8]. Resting cells challenged with a transport substrate would use a portion of the steady-state protonmotive force for uptake of the solute, requiring an increase in respiration to replenish the electrochemical proton gradient. In confirmation, non-metabolizable substrates of the lactose-transport system produced an increase in respiration (fig.2). The lack of stimulation of respiration reported [1] may reflect in part the difference in [TMG] used (2 mM vs 10 mM here) and in part the difference between the K12 strain used in that study and the ML strain used here.

Proton uptake via the F_0F_1 during ATP synthesis probably represents the other major mechanism for utilization of the protonmotive force [9]. In the absence of ATP synthesis, proton re-entry via the F_0F_1 is slow [9]. In a mutant lacking the F_1 the F_0 functions as an unregulated proton channel, resulting in a highly proton-permeable cytoplasmic membrane [4,5]. As expected, the RCI value in this mutant is greatly reduced compared to the parent strain. Strain NR71 is isogenic with NR70 except for a secondary mutation which renders the F_0 unable to translocate protons in the absence of a functional F_1 [6]. Since strains 7 and NR71 exhibit similar RCI values, the loss of respiratory control in strain NR70 is most likely due to the increase in proton movement through the F_0.

Thus, the ability to limit respiration is an advantage when the needs for energy consumption are low, such as in the resting state. When the cell is actively growing, restrictions on the respiratory rate would be unnecessary and might even be disadvantageous, since the formation of the protonmotive force is probably not the rate-limiting factor in growth.

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