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Biochimica et Biophysica Acta, 1365 (1998) 220–224

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Transcriptional regulation and energetics of alternative respiratory pathways in facultatively anaerobic bacteria

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Received 27 January 1998; received in revised form 16 March 1998; accepted 16 March 1998

Abstract

The facultatively anaerobic *Escherichia coli* is able to grow by aerobic and by anaerobic respiration. Despite the large difference in the amount of free energy that could maximally be conserved from aerobic versus anaerobic respiration, the proton potential and $\Delta G'_{Phos}$ are similar under both conditions. O_2 represses anaerobic respiration, and nitrate represses fumarate respiration. By this the terminal reductases of aerobic and anaerobic respiration are expressed in a way to obtain maximal H^+/e^- ratios and ATP yields. The respiratory dehydrogenases, on the other hand, are not synthesized in a way to achieve maximal H^+/e^- ratios. Most of the dehydrogenases of aerobic respiration do not conserve redox energy in a proton gradient whereas the enzymes from anaerobic respiration do so. Thus transcriptional regulation of the respiratory pathways by electron acceptors has multiple effects on cellular energetics. The transcriptional regulation in response to O_2 is effected by two transcriptional regulators, ArcA/B (aerobic respiratory control) and FNR (fumarate nitrate reductase regulator). FNR contains an O_2-sensitive $[4Fe–4S]^{2+}$ cluster in the sensory domain and is converted to the transcriptional inactive state in the presence of (cytoplasmic) O_2. © 1998 Elsevier Science B.V.

Keywords: Aerobic and anaerobic respiration; Proton potential; Transcriptional regulation; Regulation of energetics; Fumarate nitrate reductase regulator; O_2-sensing

1. Introduction

Facultatively anaerobic bacteria like *Escherichia coli* are able to use nitrate, fumarate and dimethylsulfoxide (DMSO) as acceptors for respiration if no oxygen is available, or to gain energy by fermentation. The switch from aerobic to anaerobic catabolism has many consequences on cellular energetics and requires the presence of O_2 and nitrate sensitive transcriptional regulators to adapt the expression of the respective genes [7,10,11,20,24–26]. In *E. coli* two O_2-responsive transcriptional regulators are known, ArcA/B (*a*erobic *r*espiratory *c*ontrol) [11] and FNR (*f*umarate *n*itrate reductase *r*egulator) [10,20,24,25], which control expression of the respective genes in response to O_2. The switch from aerobic to anaerobic respiration (and fermentation) has important consequences on cellular energetics and ATP yields, since the free energy differences for the anaerobic respiratory chains are distinctly lower than those for the aerobic [10,24–26]. It turned out that the synthesis of the terminal reductases is regulated in a way to synthesize the enzymes with maximal ATP yields under the respective growth condition [10,11,24]. The synthesis of alternative respiratory dehydrogenases, on the other hand, often is not

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PII: S0005-2728(98)00071-1

regulated such as to obtain maximal ATP yields [22,25]. Thus understanding the rationale of transcriptional regulation by environmental signals like O_2 and nitrate is important for the understanding of cellular energetics.

2. Energetics of growth by aerobic and anaerobic respiration in facultatively anaerobic bacteria

In the absence of oxygen, less energy can be produced from oxidation of carbon sources during anaerobic respiration due to the different redox potentials of the terminal acceptors [22,24,25]. The free energy ($\Delta G^{0\prime}$) for glucose oxidation with O_2, for example, is up to 13-fold higher than for fermentation or anaerobic respiration (Table 1). Similarly, $\Delta G^{0\prime}$ for respiration with NADH decreases from −233 kJ/mol NADH in aerobic respiration to −67 kJ/mol in fumarate respiration [10,24,25]. Nevertheless, the energetic parameters of *E. coli* cells do not change largely upon aerobic/anaerobic transition (Table 1) [23]. Under steady state conditions of aerobic and anaerobic respiration and fermentation, the phosphorylation potential stays constant at 47 kJ/mol. The proton potential of −160 mV during aerobic respiration decreases only slightly during anaerobic respiration irrespective of the midpoint potential of the acceptor. Only in fermentation a more significant decrease in Δp is observed (Table 1). Due to the very similar energetic situation processes depending on Δp such as ATP-synthesis or transport of solutes across membranes can function in a similar way under conditions of aerobic and anaerobic respiration [23].

For the Δp values given in Table 1 the H^+/ATP ratios for ADP phosphorylation are in the range from 3.1 to 3.6 for aerobic and anaerobic respiration, compatible with a H^+/ATP ratio of 4 found for plant type F_0F_1 ATPase [28]. A supposed decrease of Δp to about −100 mV in anaerobic respiration of *E. coli* as suggested earlier, requires an increase of the H^+/ATP ratio to a value of 5 for ADP phosphorylation in anaerobic respiration, which is unlikely. Thus it is obvious that *E. coli* maintains $\Delta G'_{Phos}$ and Δp at rather constant levels during aerobic and anaerobic respiration despite large differences in ΔE of the respiratory chains. The same applies also to the anaerobic bacterium *Wolinella succinogenes*, which is able to grow by respiration with electropositive (nitrate, $E^{0\prime}=+420$ mV, or fumarate, $E^{0\prime}=+30$ mV) and electronegative acceptors (polysulfide, $E^{0\prime}=-260$ mV). With both types of acceptors (and H_2 or formate as the donor) Δp maintains constant at about −170 mV [14,19].

3. Synthesis of terminal reductases, but not of the respiratory dehydrogenases is optimized for maximal ATP yields

In *E. coli* and other facultatively anaerobic bacteria the synthesis of respiratory enzymes is regulated at the transcriptional level by regulators responding to O_2 and nitrate [10,11,24–27]. The oxidases and terminal reductases are expressed in a hierarchical way, i.e., oxygen represses fermentation and anaerobic respiration with nitrate or fumarate, and nitrate prevents fermentation and fumarate respiration by repression of the respective structural genes. The

Table 1
Energetic parameters of *E. coli* for growth by aerobic and anaerobic respiration and by fermentation

Acceptor for respiration	$\Delta G^{0\prime}$ (kJ/mol Gluc)	$E^{0\prime}$ (mV)	$\Delta G'_{Phos}$ [a] (kJ/mol)	Δp [a] (mV)	m_{min} (H^+/ATP)
O_2	−2830	+820	47.7	−160	3.1
Nitrate	−858	+420	46.2	−140	3.3
DMSO	−650	+160	47.2	−137	3.6
Fumarate	−550	+30	47.6	−145	3.4
–(Fermentation)	−218	–	46.5	−117	–

[a] Measured with glycerol as the C-source, except for fermentation (glucose).
The $\Delta G^{0\prime}$ values refer to growth on glucose and the respective acceptors [24], the values for $\Delta G'_{Phos}$ and Δp are taken from Ref. [23]. The number of H^+ required for ATP synthesis by ATP-synthase (m_{min}) was calculated from $\Delta G'_{Phos} \leq m \cdot F \cdot \Delta p$ [23].

enzymes associated with the corresponding respiratory chains have different H^+/e^- ratios and amount to 2, 1, and 0 for quinol oxidase *bo* (Cyo), nitrate reductase (NarG) and fumarate reductase (Frd), respectively (see Refs. [7,25]). The ATP yields are thus highest for the oxidases, intermediate for nitrate reductase, and lowest for fumarate reductase which could be the regulatory rationale for the observed hierarchy in regulation. In other bacteria, however, electron acceptors with high ATP yields are not used preferentially. In *W. succinogenes* the most electronegative acceptor (polysulfide) with the lowest ATP yields, represses nitrate and fumarate respiration which both give higher ATP yields [15].

Many of the respiratory dehydrogenases of *E. coli* are transcriptionally regulated by oxygen and nitrate, too, and in aerobic and anaerobic respiration different dehydrogenases are used [5,9,22,25,27]. Most of the dehydrogenases which are synthesized under aerobic conditions apparently are not able to couple the redox reaction to the generation of a proton potential, although the redox reactions could supply sufficient free energy for H^+ translocation. This applies to glycerol-3-P (GlpD), NADH (Ndh), lactate (Dld, LctD), D-amino acid (DadA) dehydrogenases and pyruvate oxidase (PoxB) (Fig. 1) (see Ref. [25]). On the other hand, dehydrogenases which are mainly involved in anaerobic respiration, like NADH (NuoA-N), glycerol-3-P (GlpA) and formate (FdnG) dehydrogenases or hydrogenase (HybABC) are known, or supposed, to couple the redox reaction to H^+ translocation (Fig. 1) [4,22,25]. This principle becomes most obvious for the isoenzymes of dehydrogenases which are present in the bacteria (Fig. 1): the coupling isoenzymes (NuoA-N and presumably GlpA) operate in anaerobic respiration, the noncoupling enzymes (Ndh and GlpD) are the major enzymes in aerobic respiration [6,22,25]. The requirement for the coupling enzyme in (anaerobic) fumarate or DMSO respiration is obvious, since the enzyme provides the only site for H^+ translocation. Under an energetic point of view, the NADH:quinone oxidoreductase reaction could be coupled to energy conservation in aerobic respiration as well, and the same applies to the use of the glycerol-3-phosphate dehydrogenase isoenzymes under oxic and anoxic conditions. Thus, it appears that most of the dehydrogenases of aerobic respiration do not conserve redox energy in a proton gradient, whereas most dehydrogenases of anaerobic respiration do so [25,27]. This causes H^+/e^- ratios or ATP yields in aerobic respiration which are distinctly below the values which could be achieved (Table 2) [6,25]. This indicates that high metabolic flux rates which are stimulated by low coupling coefficients, are important in aerobic growth when ATP yields are not limiting. A similar principle can be observed for other pathways of *E. coli*, too, which are not expressed in sufficient amounts to achieve optimal ATP yields [17].

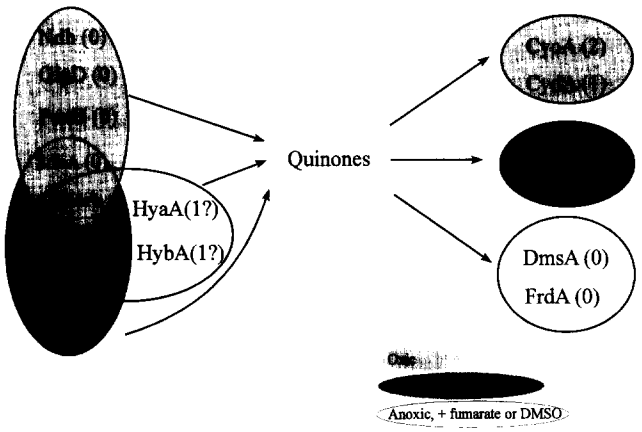

Fig. 1. Dehydrogenases and terminal reductases of aerobic and anaerobic respiration of *E. coli*. The figure gives the conditions (i.e., presence of electron acceptors) for the synthesis of the respiratory dehydrogenases and terminal reductases of the respective respiratory chains. The data are obtained from expression studies, analysis of mutants and measurement of enzyme activities. For any condition only the major enzyme present is considered. For each of the enzymes the H^+/e^- ratio is given in brackets. For more details see Ref. [25].

Table 2
Variation in H^+/e^- ratios for aerobic and fumarate respiration in *E. coli* (NADH as donor) by the use of alternative isoenzymes

Reaction	Enzymes	H^+/e^-	Comment
NADH→O_2	Nuo+Cyo	2+2=4	
	Nuo+Cyd	2+1=3	
	Ndh+Cyo	0+2=2	Major path
	Ndh+Cyd	0+1=1	
NADH→fumarate	Nuo+Frd	2+0=2	Major path
	Ndh+Frd	0+0=0	

The isoenzymes are NADH dehydrogenase I (Nuo) or II (Ndh), or quinol oxidases *bo* (Cyo) and *bd* (Cyd). The H^+/e^- ratios give the ratios for the individual enzymes and the complete path. For references, see Ref. [25].

4. Regulators and signals controlling the synthesis of respiratory enzymes in response to electron acceptors

Transcriptional regulation of the genes of aerobic and anaerobic respiration is effected mainly in response to O_2 and nitrate, but also to the type of the C-source and the growth phase. For the O_2 regulated genes defined O_2 tensions for half-maximal expression ($pO_{0.5}$) can be determined in an oxystat [1,2,26]. The *sdh* genes encoding succinate dehydrogenase of aerobic respiration are expressed efficiently only at high oxygen tensions (above 5 mbar O_2), whereas the genes or metabolic pathways of microaerobic respiration (between 1 to 5 mbar), anaerobic respiration (below 5 mbar) or fermentation (below 1 mbar) are expressed or functional at distinctly lower oxygen tensions. Thus the corresponding metabolic systems are functional in succession with decreasing pO_2 (aerobic respiration> microaerobic respiration>anaerobic respiration> fermentation).

Regulation by O_2 is effected by the O_2-sensing transcriptional regulators FNR and ArcA/B [11,20], regulation by nitrate by the sensor-regulators NarX/L and NarP/Q [21]. The latter are two-component regulatory systems consisting of a membraneous sensory kinase (ArcB, NarX, NarQ) and a cytoplasmic response regulator (ArcA, NarL, NarQ) (for reviews see Refs. [11,21]). The second O_2-sensor, FNR, is a 'one-component' sensor-regulator consisting of a sensory and of a regulatory domain within the same protein [20,26]. The protein is located in the cytoplasm of the bacteria and is assumed to react there directly with molecular oxygen. It has been shown that under aerobic and microaerobic conditions the cytoplasm of bacteria is rich in O_2 [29] due to the rapid diffusion of oxygen and the small cell dimensions [1,26,27]. Thus there is sufficient O_2 present in the cytoplasm for direct reaction with FNR. Only at very low external O_2 tensions ($pO_2<1$ mbar) the cytoplasm might become anoxic in accordance with the relevant regulatory O_2 tensions of FNR.

The sensory domain of FNR consists of a Fe–S cluster which is of the $[4Fe–4S]^{2+}$ type under anoxic conditions [3,8,12,16,26,27]. Then the protein is in the active state and activates or represses target genes. The cluster is liganded by four Cys-residues, three of which (Cys_{20}, Cys_{23}, Cys_{29}) are located in the N-terminal end, the fourth (Cys_{122}) in the central part of the protein. It is suggested that the cytoplasmic oxygen reacts with FNR by direct interaction [1,26,27,30]. In vitro, the Fe–S cluster is converted to a $[2Fe–2S]^{2+}$ cluster by oxygen, resulting in FNR inactivation [13]. The significance of a $[3Fe–4S]^{+}$ cluster which was observed after incubation of FNR with oxygen is not clear [8,12]. After prolonged exposure to oxygen, the Fe–S cluster is destroyed. Reassembly of the [4Fe–4S] cluster might require cellular proteins such as the NifS-like protein of *E. coli* [8,12,27].

Acknowledgements

Work in the authors laboratory was supported by Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie

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