The role of antioxidant enzymes in response of *Escherichia coli* to osmotic upshift

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**Abstract**

Aerobic growth of *Escherichia coli* sodAsodB and katE mutants lacking cytosolic superoxide dismutases and catalase hydroperoxidase II was inhibited by osmotic upshift to a greater extent than of their wild-type parent strains. The fur mutation leading to an intracellular overload of iron also increased sensitivity of growing *E. coli* cells to osmotic upshift. Using lacZ fusions, it was shown that expression of antioxidant genes soxS and katE was stimulated by an increase in osmolarity. These data suggest that in aerobically growing *E. coli* cells, moderate osmotic upshift causes activation of certain antioxidant systems. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Osmotic upshift; Antioxidant enzyme; *Escherichia coli*

1. Introduction

The reactive forms of oxygen such as H$_2$O$_2$, superoxide anion (O$_2^-$) and hydroxyl radicals are produced by both environmental agents and normal aerobic metabolism. These reactive oxygen species can damage DNA, proteins and membrane lipids and can result in mutagenesis, inhibition of growth and cell death [1].

*Escherichia coli* possesses several defense systems that may protect cells from oxidative damage [2,3]. These systems can be induced in response to oxidative stress caused by imbalances between the production and detoxification of oxygen radicals. In growing *E. coli* cells, the oxyR system is induced in response to H$_2$O$_2$. This regulon includes catalase hydroperoxidase I (HPI), encoded by katG, an NADPH-dependent alkyl hydroperoxidase, encoded by ahpFC, glutathione reductase, encoded by gorA, and several other genes [2,3]. Catalase HPI is controlled by not only oxyR, but also rpoS [4]. The two-stage soxRS system is activated by exposure of aerobically growing *E. coli* to compounds that generate intracellular superoxide, such as menadione or paraquat. Control of the soxRS system is effected by the products of the soxR and soxS genes. This regulon includes Mn-containing superoxide dismutase (MnSOD), encoded by sodA, DNA repair endonuclease IV, encoded by nfo, glucose-6-phosphate dehydrogenase (G6PD), encoded by zwf [2,3].

In addition to H$_2$O$_2$-inducible catalase HPI, *E. coli* produces the monofunctional catalase HPII, encoded by katE. HPII synthesis is induced during entry into the stationary phase and its expression is controlled by the σ subunit of RNA polymerase, encoded by the rpoS (katF) gene [5].

It was shown that under different stresses unrelated to a direct action of oxidants, the induction of various antioxidant enzymes was observed. In *E. coli*, the aerobic heat shock induces MnSOD [6], and response to nutrient depletion is associated with an increase in catalase HPII [5]. In this study, we investigated the role of antioxidant enzymes in response of *E. coli* to osmotic upshift.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The *E. coli* strains used in this study are listed in Table 1. All strains were grown overnight at 37°C in M9 medium [7] supplemented with 0.2% glucose, 0.2% casamino acids...
Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>GC4468</td>
<td>ΔlacU169 rpsL soxRS⁺</td>
<td>[11]</td>
</tr>
<tr>
<td>QC909</td>
<td>as GC4468 but (socA::Mu dPr13)25 (socB::kan)/Al-2</td>
<td>[11]</td>
</tr>
<tr>
<td>QC772</td>
<td>as GC4468 but Δfar::kan</td>
<td>[15]</td>
</tr>
<tr>
<td>MN101</td>
<td>as GC4468 but katG::lacZ</td>
<td>This study</td>
</tr>
<tr>
<td>MN111</td>
<td>as GC4468 but katG::lacZ</td>
<td>This study</td>
</tr>
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(CA), 1 μg of thiamine per ml and the appropriate antibiotics. Glucose–amino acid medium was identical to the glucose minimal medium, except that it was supplemented with 20 amino acids at 0.5 mM. After centrifugation of overnight cultures, bacteria were resuspended in the same fresh medium (100 ml in a 250 ml flask) and were cultivated at 37°C with rotary shaking (150 rpm) in all cases without antibiotics. Growth was monitored by measuring the absorbance at 670 nm. To determine the response of E. coli to osmotic stress, the osmolarity of the M9 medium was increased by the addition of granular sucrose or NaCl. Osmolytes were added in the middle of the exponential phase, when cell density was 0.5 g dry cells l⁻¹.

2.2. Strain constructions

Plasmid DNA preparation and transformation protocols were done by standard procedures as previously described [7]. The plasmid pRSkatE16 carrying the katE::lacZ fusion [4] was transformed from AI1326 into GC4468. The plasmid pKT1033 carrying the katG::lacZ fusion [8] was transformed from RK4936 into GC4468. Transformants were selected on LB plates containing ampicillin and streptomycin. In addition, transformants were tested for β-galactosidase activity.

2.3. Enzyme assays

β-Galactosidase activity was determined by the method of Miller [7] and normalized to the cell density. G6PD activity was assayed by following the reduction of NADP at 340 nm [9]. Protein concentration was determined by the Lowry method with bovine serum albumin as a standard [10].

3. Results

In order to assess the role of MnSOD and FeSOD in response of E. coli to osmotic upshift, mutant strain QC909 (socA::lacZ) that lacks both cytosolic SODs [11] was used. Media with elevated osmotic strengths were attained by adding sucrose or NaCl. Fig. 1 shows the relation of the specific growth rate to the osmolality of medium for QC909 and GC4468 (wild-type). The SOD-deficient cells revealed higher sensitivity to an osmotic upshift than the wild-type. The effect of socA::lacZ mutation was more pronounced at higher concentrations of both osmolytes (above 0.4 mM).

Imlay and Fridovich reported that growth of socA::lacZ mutant E. coli J1132 growing in aerobic minimal medium supplement with 20 amino acids can be stimulated by the presence of 0.1–0.3 M extracellular osmolytes [12]. Under our conditions, osmotic upshift with 0.2 M sucrose or 0.3 M NaCl resulted in a moderate reduction in the growth rate of socA::lacZ mutant QC909 growing in minimal medium supplement with CA (Fig. 1) or 20 amino acids (data not shown). It is possible that this is due to the peculiarities of growth medium and the strains used in studies. However, it is most likely that contradiction between our results and those of Imlay and Fridovich is due to distinct experimental conditions. Imlay and Fridovich measured the growth rate for at least five generations, starting with a very small cell density. Cells were grown in medium with an elevated osmolarity throughout the experiment [12]. Under these conditions, the bacterial adaptation to osmotic stress must occur. In our study, the growth rate was measured within 2 h after the abrupt osmotic upshock (1–2 generations). Osmolytes were added in the middle of the exponential phase, when cell density was 0.5 g dry cells per l.

It is possible that the elevated sensitivity to an osmotic upshift of the socA::lacZ mutants results from an increase in intracellular levels of O₂⁻. If this is the case, the induction of the superoxide-inducible genes by an osmotic upshift may be observed. We explored this possibility by assaying β-galactosidase activity in E. coli strains TN521 and QC772 containing socA::lacZ and soxS::lacZ operons, respectively [11,13]. It has been shown that socA and soxS genes are induced by superoxide-generating agents [3]. In fact, the addition of 0.4 or 0.9 mM sucrose to growing TN521 led to markedly increased expression of soxS::lacZ (Fig. 2). After addition of 0.3 M NaCl, soxS::lacZ expression increased 2-fold. socA::lacZ expression was also 2-fold higher in the presence of 0.4 M sucrose than in the absence of osmolyte (data not shown).
The latter result is in agreement with the observation that osmotic shock by 0.5 M NaCl results in a 2–3-fold increase in sodA expression [14].

G6PD is a further enzyme induced by an increase in the intracellular O$_3^2$ and controlled by soxRS [3,9]. The addition of 0.4 mM sucrose had no significant effect on the activity of G6PD in E. coli GC4468.

It is known that under aerobic conditions, intracellular free iron potentiates oxidative stress promoting the production of highly deleterious hydroxyl radicals in the Fenton reaction. It has been reported that the deregulation of iron assimilation in E. coli fur mutants causes an oxidative stress due to iron overload [15]. When E. coli QC1732 (Δfur) was grown in a high-osmolarity medium containing 0.6 M NaCl, its growth rate decreased more drastically than in parental strain GC4468 under the same conditions (Fig. 3). A similar response of E. coli QC1732 was also observed when the medium osmolarity was increased by the addition of 0.6 M sucrose. The addition of osmolyte resulted in a decrease in the specific growth rate of the fur mutants and parental strain to 28 and 45%, respectively. However, adding of the iron chelator 1,10-phenantroline (0.025–0.1 mM) to both cultures did not significantly change their response to the osmotic stress.

In exponentially growing E. coli, exposure to low concentrations of O$_3^2$-generating agents or H$_2$O$_2$ increases resistance to much higher levels of these oxidants [2,3]. An increase in the intracellular level of O$_3^2$ occurs under osmotic stress, so it may be expected of changes in osmotolerance of cells pretreated with low doses of O$_3^2$-generating agents. However, pretreatment with low concentrations of iron assimilation in E. coli fur mutants causes an oxidative stress due to iron overload [15]. When E. coli QC1732 (Δfur) was grown in a high-osmolarity medium containing 0.6 M NaCl, its growth rate decreased more drastically than in parental strain GC4468 under the same conditions (Fig. 3). A similar response of E. coli QC1732 was also observed when the medium osmolarity was increased by the addition of 0.6 M sucrose. The addition of osmolyte resulted in a decrease in the specific growth rate of the fur mutants and parental strain to 28 and 45%, respectively. However, adding of the iron chelator 1,10-phenantroline (0.025–0.1 mM) to both cultures did not significantly change their response to the osmotic stress.

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aCells were grown to the middle of the exponential phase aerobically in conditions. Pretreatment with 60-200 more likely it enhanced growth inhibition under these conditions. It is notable that in the reciprocal situation, growth of growing cells in response to moderate osmotic upshift [16]. One of a number of genes, may contribute to the prevention of oxidative stress. Results of this study indicate that the expression of the katE gene controlled by rpoS increases in response of growing E. coli to osmotic shock. These data confirm the role of a as a global regulator in the osmotic control of gene expression not only in early stationary phase cells but also in exponentially growing cells in response to moderate osmotic upshift [16].

4. Discussion

A sudden increase in external osmolarity results in plasmolysis accompanied by an inhibition of growth and DNA replication [17,18]. Adaptive responses to osmotic stress involve the accumulation of solutes, such as potassium ions or glycine betaine, and the increased synthesis of metabolites like glutamate and trehalose [17,18]. An osmotic upshift also results in inhibition of different transport systems and respiration. It has been proposed that inhibition of electron transfer chain and other membrane functions could result from conformational changes of the different membrane proteins induced by the deformation of the membranes [19]. It is probably that disturbance of the membrane functions induced by an osmotic upshift may result in an increase in the production of the active oxygen species by the respiratory chain. It was reported that the membrane components of the respiratory chain can be the sources of oxygen free radicals in aerobically growing E. coli cells [20,21].

The enhanced sensitivity of the sodAsodB and Δfur strains to an osmotic upshift and the osmotic induction of soxS:::lacZ and sodA:::lacZ suggest that in growing aerobic E. coli, an osmotic upshift can be accompanied by an increase in the production of active oxygen species. In wild-type strains, osmotic induction of antioxidant systems, controlled by soxRS and rpoS genes, may contribute to the prevention of oxidative stress.

Previously, it had been shown that mutant rpoS (katF) exhibited greater sensitivity to osmotic shock during growth and starvation [22]. Results of this study indicate that the expression of the katE gene controlled by rpoS increases in response of growing E. coli to osmotic shock.

These data confirm the role of a as a global regulator in the osmotic control of gene expression not only in early stationary phase cells but also in exponentially growing cells in response to moderate osmotic upshift [16].

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