Regulatory locus soxRS partially protects Escherichia coli against ozone

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Received 24 October 2000; received in revised form 18 December 2000; accepted 20 December 2000

Abstract

Ozone is one of the major city air pollutants. Since it is known to induce the overexpression of superoxide-dismutase in various models, and is also a powerful oxidant, we tested if ozone can induce the expression of the soxRS regulon of Escherichia coli, which is activated by superoxide and nitric oxide. A sub-lethal exposure to ozone was unable to activate the expression of soxS: :lacZ transcriptional fusions. However, cells lacking the soxRS locus were more susceptible than wild-type to ozone-mediated killing. Constitutive expression of the soxRS regulon did not increase the resistance to ozone. Ozone might be exerting a selective pressure upon oxidative-stress defense mechanisms in airborne bacteria. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Escherichia coli; Ozone; soxRS locus

1. Introduction

Ozone (O3) is a very unstable and powerful oxidizing compound. It is continuously produced in the upper atmosphere by the action of solar ultraviolet radiation upon air dioxygen (O2). However, at low altitudes, it is one of the most important and damaging air pollutants; it is generated through a series of photochemical reactions, involving hydrocarbon molecules from the incomplete combustion of fuels used by industry and automobiles [1]. In Mexico City, for instance, O3 concentration in the air is often above 0.11 ppm (the National Ambient Air Quality Standard of the USA) [2], and can even reach concentrations as high as 0.3 ppm. When exposed to O3, starting around 0.1 ppm, a number of effects have been observed in humans, such as eye, nose and throat irritation, headache, and pulmonary function decrement [3]. Ozone also has extensive genotoxic effects [4].

Ozone has been used as a disinfectant, both for air and water [4]; however, the molecular effects of O3 exposure upon bacteria have not been very extensively explored.

Sub-lethal doses are known to induce the expression of sodA, encoding a Mn-superoxide dismutase [5]. sodA is a multi-regulated gene, whose expression is activated, among other conditions, by the intracellular generation of superoxide (O2−) and the extracellular presence of NO•; this activation is mediated by the soxRS regulon genes. This is a mini-cascade regulatory system [6], where SoxR induces the expression of soxS in cells under oxidative stress [7,8]; SoxS protein, in turn, induces the expression of the genes of the soxRS regulon [9]. These include, in addition to sodA, another > 15 genes for defense and repair functions, the expression of which confers resistance to oxidants and antibiotics; the soxRS regulon extensively overlaps with the marRAB regulon [10]. Here, we report that an ozone exposure capable of inducing the expression of sodA, does not induce the expression of a soxS: : lacZ fusion; furthermore, sodA expression is induced even in the absence of soxRS genes.

2. Materials and methods

Escherichia coli strains GC4468 (soxR+), JTG1052 (soxR101, constitutive) and DJ901 (ΔsoxRS) [11], and TN521 (soxR+) and TN531 (ΔsoxRS) carrying a soxS: : lacZ fusion [12] were used. Also, DJ901 cells carrying either plasmid pSXR or plasmid pSXS independently...
(pSE380 carrying genes soxR or soxS, respectively, under the control of a tac promoter [13]). Cells were cultured in LB medium.

To test for soxRS activation by ozone, under conditions similar as to exposure of airborne bacteria to polluted air, strains TN521 and TN531, were cultured to mid-exponential phase, deposited (~10^8 CFU) on 22-mm membrane-filter disks (Millipore) and allowed to air-dry. They were then exposed to ozone, 0.3 ppm, generated by an electric-arc device (Puraqua, Mexico City, Mexico), measured with an Ozone Sensor (Eco Sensors, Santa Fe, NM, USA). Immediately after a 30-s exposure, the disks were placed for 30 min on LB-agar plates and incubated at 35°C. The disks were then soaked and vortexed in Z buffer to release the cells, and the β-galactosidase activity of the recovered cells was assayed as described by Miller [14]. As a positive control, 500 nmol paraquat (a redox-cycling compound known to activate the control, 500 nmol paraquat (a redox-cycling compound known to activate the soxRS regulon [11]) was added to a set of disks, instead of O3 treatment. A similar ozone treatment was also applied to GC4468 and DJ901 cells, after which cells were disrupted using a Bead-Beater, and the crude extract applied to a superoxide-dismutase-activity gel, as described by Beauchamp and Fridovich [15].

To assess the soxRS-related susceptibility to ozone, strains GC4468, JTG1052 and DJ901, and DJ901 carrying plasmids pSE380, pSXR or pSXS, were also spotted on filter disks (~10^5 CFU) and exposed to 0.6 ppm ozone during 1–5 min. The disks were then placed on LB-agar plates and incubated for 24 h at 35°C. The minimal exposure time that killed all the organisms was recorded.

3. Results and discussion

A low-level ozone exposure, similar to the one receiving an airborne bacteria in a polluted city, is incapable of inducing the expression of a soxS′::lacZ fusion (Table 1). Higher concentrations did not induce the expression of the fusion, and the recovery of viable cells is greatly diminished (data not shown). However, the expression of sodA is elicited by 0.3 ppm ozone, although it seems to be independent of soxRS since it also occurs in a ΔsoxRS strain (Fig. 1). The induction of sodA by ozone has been previously reported [5]. This effect can therefore be attributed to other regulatory systems; sodA expression is also controlled by Fur (amongst other regulatory proteins), depending on iron availability [16]. By disrupting membranes, ozone might be changing redox potential and/or releasing iron pockets, affecting the Fe^{3+}/Fe^{2+} balance; the toxicity of menadione/ascorbate treatment has also been attributed to membrane-related effects [17].

Strain DJ901 was more sensitive than GC4468, but no additional resistance was observed in strain JTG1052, indicating that constitutive expression of soxRS-regulon genes does not provide additional protection against ozone (Table 2). Furthermore, cells bearing pSXS were able to withstand a longer exposition to ozone than those carrying pSXR or just the vector plasmid. However, overexpression of SoxS, caused by IPTG-treatment of pSXS-carrying cells, did not extend their survival (Table 2). These results indicate that the basal expression of soxRS-regulon genes can protect cells against ozone toxicity, but prior continuous expression of the regulon does not exert further protection. Mn-SOD is a likely candidate for this protective effect, since SOD has been reported to protect bacterial cells from ozone exposure [18].

However, it seems likely that ozone, as an air pollutant, can be selecting for cells bearing sox genes, which allows for increased survival in the presence of this agent. Therefore, the effect of ozone in promoting airborne infections

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>β-Galactosidase activity (Miller units)</th>
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<tbody>
<tr>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>TN521</td>
<td>326</td>
</tr>
<tr>
<td>TN531</td>
<td>281</td>
</tr>
</tbody>
</table>

*Average of three independent experiments (standard deviation ± 10%); PQ, paraquat.

Table 2

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Pre-treatment</th>
<th>Killing time (min) ± S.D.*</th>
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</thead>
<tbody>
<tr>
<td>GC4468</td>
<td>–</td>
<td>4.5</td>
</tr>
<tr>
<td>DJ901</td>
<td>–</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>JTG1052</td>
<td>–</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>DJ901/pSE380</td>
<td>–</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>DJ901/pSXS</td>
<td>IPTG</td>
<td>4.0</td>
</tr>
<tr>
<td>DJ901/pSXS</td>
<td>–</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>DJ901/pSXR</td>
<td>IPTG</td>
<td>3.0</td>
</tr>
<tr>
<td>DJ901/pSXR</td>
<td>–</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Minimal exposure resulting in killing all spotted cells; cells were exposed from 1 to 5 min, in 0.5-min steps; where no S.D. value is shown, results of all three independent experiments were identical.
may be two-fold: by eroding the integrity of respiratory mucosa and impairing other defense mechanisms, and independently by selecting bacteria more able of withstanding the ‘oxidative burst’ of macrophages [19,20] and perhaps even antibiotics. The role of soxRS in increasing antibiotic resistance, not only in response to oxidants, but also to other environmental pollutants, such as mercury, has also been described [21].

Acknowledgements

This work was made possible by a Fogarty International Research Collaboration Award from the NIH (P.I., Bruce Demple); we thank him for his extensive help. Fig. 1 was prepared by Isabel Nivón-Bolán.

References