Effects of Overproduction of Superoxide Dismutase on the Toxicity of Paraquat toward Escherichia coli*

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(Received for publication, December 13, 1990)

Gross overproduction of the manganese-containing superoxide dismutase in Escherichia coli, by virtue of a multicopy plasmid bearing the sodA gene, decreases enumeration on paraquat-containing agar plates. This reflects growth inhibition, not lethality, since cells on these plates can be rescued by exclusion of dioxygen. Growth in liquid medium revealed that the control strain adapted to growth in the presence of paraquat more rapidly than did the overproducer. Glucose-6-phosphate dehydrogenase, taken as a representative of the superoxide-inducible soxR regulon, was induced during exposure to paraquat to a much greater extent in the control than in the superoxide dismutase-overproducing strain. These results support the view that overproduction of superoxide dismutase interferes with induction of the soxR regulon and thus prevents a balanced adaptation to the multiple aspects of the toxicity of aerobic paraquat.

O₂⁻ is an agent of the toxicity of dioxygen and of the dioxygen-dependent toxicities of viologens and quinones. Superoxide dismutases, which catalytically scavenge this radical, play a defensive role. This view is supported by the inductions of superoxide dismutase by dioxygen and by aerobic viologens and quinones and by the correlation between cell content of superoxide dismutase and resistance toward the toxicities of these agents (1–4). The dioxygen-dependent phenotypic abnormalities associated with mutational defects in the superoxide dismutase genes and their elimination by reintroduction of a functional superoxide dismutase gene also eloquently support this position (4–10).

Nevertheless, gross overproduction of superoxide dismutase, achieved with multicopy plasmids bearing functional superoxide dismutase genes, has been associated with increased sensitivity toward PQ⁺⁺⁺ (11, 12). This apparently paradoxical result has been attributed to increased production of H₂O₂ at the elevated levels of superoxide dismutase (12). This explanation seemed unlikely to us since the effect of superoxide dismutase would be to markedly lower the steady state level of O₂⁻, not to change the rate of production of H₂O₂ during that steady state. We have therefore examined the basis of the increased sensitivity toward PQ⁺⁺⁺ of the superoxide dismutase overproducer. We find that gross overproduction of superoxide dismutase imposes a deficit in the induction of glucose-6-phosphate dehydrogenase, which likely mirrors similar deficits in the induction of other products of the soxR (13, 14) regulon. This is the probable cause of the increased sensitivity of the overproducer toward the growth-inhibiting effect of PQ⁺⁺⁺.

RESULTS

**PQ⁺⁺ Toxicity on Plates**—Bloch and Ausubel (11) used plates containing graded concentrations of PQ⁺⁺ and noted a dioxygen-dependent decrease in enumeration with increasing concentrations of PQ⁺⁺. The manganese-containing superoxide dismutase overproducer DT was reported to be more sensitive toward this effect of PQ⁺⁺ than was the control strain HC. The methods they used would not distinguish between lethality and growth inhibition. The pH of the medium and the time of incubation, between plating and counting, are important variables since PQ⁺⁺ uptake into E. coli becomes more effective as the pH is raised (19) and, in the presence of growth inhibitors, the number of visible colonies is dependent upon elapsed time. These variables may account for the failure of other workers (2) to repeat the results of Bloch and Ausubel (11).

The data in Fig. 1, collected at pH 6.9, reflect the greater sensitivity toward PQ⁺⁺ of DT (line 1) as compared with HC (line 2), in agreement with Bloch and Ausubel (11). This
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Fig. 1. Decrease in enumeration as a function of paraquat. DT (line 1) and HC (line 2) were plated on Luria broth agar + 50 μg/ml ampicillin and the indicated concentrations of paraquat at pH 6.9. Colonies were counted after 48 h of aerobic incubation at 37°C.

![Graph showing decrease in enumeration as a function of paraquat concentration.](image)

difference was also apparent in colony morphology. Thus, on plates containing PQ++, HC produced much larger colonies than did DT. This difference in colony morphology suggested that the difference between HC and DT lay in susceptibility to the growth-inhibiting effect of PQ++, rather than to any difference in lethality.

This was further explored at pH 7.5. The data in Fig. 2 (lines 1 and 2) again show that DT was more sensitive to PQ++ than was HC, and once again the DT colonies were smaller than the HC colonies. When plates were incubated aerobically for 6 h (lines 3 and 4), or for 24 h (lines 5 and 6), and then incubated anaerobically for an additional 48 h, the effect of PQ++ on enumeration was largely eliminated. Moreover, HC and DT then gave colonies of comparable size. This demonstrates that the effect of PQ++ on enumeration, as performed by Bloch and Ausubel (11) and as repeated here, was very largely due to inhibition of growth. There was some loss of viability during 24 h of aerobic incubation on plates containing PQ++, as shown by the differences between lines 3 and 4 and lines 5 and 6, but the extent of this cell killing by PQ++ was the same with HC as it was with DT.

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**PQ++ Toxicity in Liquid Cultures**—The greater susceptibility of DT, compared with HC, to the growth-inhibiting effect of PQ++, at pH 6.9, is shown in terms of A_{600nm} in Fig. 3. It is particularly interesting that this difference became most pronounced after 3 h of incubation, as though HC was gaining resistance toward PQ++ more rapidly than was DT. This point was underscored when this experiment was repeated at pH 7.5, as shown in Fig. 4. In this case, HC escaped from the inhibitory effect of 50 μM PQ++ after 6 h, whereas DT did not. At 200 μM PQ++, neither HC nor DT escaped from growth inhibition during the 10 h of observation.

The basis of this escape from the inhibitory effect of PQ++ was explored. The cells were incubated with 50 μM PQ++, as in lines 3 and 4 of Fig. 4, for 4 h and were then diluted 20-fold into fresh medium with or without 50 μM PQ++. The results are shown in Fig. 5. After 20-fold dilution with PQ+-free medium, DT (line 2) grew almost as well as HC (line 1). Dilution with PQ++-containing medium, on the other hand, revealed that HC (line 3) was already partially adapted to PQ++, whereas DT (line 4) was not. Thus, as shown by lines 3 and 4 in Fig. 4, HC did not begin to outgrow DT in 50 μM PQ++ until 6 h had elapsed, whereas it did so at 3 h (lines 3 and 4 in Fig. 5) when diluted with fresh PQ++-containing medium after 4 h of preincubation in that medium. It thus appears that HC can adapt to PQ++ more readily than can
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Fig. 5. Adaptation by preincubation with paraquat. DT (lines 2 and 4) and HC (lines 1 and 3) were exposed for 4 h at 37 °C to 50 μM paraquat in the liquid medium and were then diluted 20-fold into fresh medium with (lines 3 and 4) or without (lines 1 and 2) 50 μM paraquat.

Table 1

<table>
<thead>
<tr>
<th>E. coli</th>
<th>PQ**</th>
<th>Mn(II)</th>
<th>Glucose-6-phosphate dehydrogenase</th>
<th>Superoxide dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
<td>units/mg</td>
<td>%</td>
</tr>
<tr>
<td>HC</td>
<td>0</td>
<td>0</td>
<td>0.078 100</td>
<td>35.9 100</td>
</tr>
<tr>
<td>DT</td>
<td>0</td>
<td>0</td>
<td>0.077 99</td>
<td>89.1 250</td>
</tr>
<tr>
<td>HC</td>
<td>50</td>
<td>0</td>
<td>0.647 800</td>
<td>137 380</td>
</tr>
<tr>
<td>DT</td>
<td>50</td>
<td>0</td>
<td>0.201 250</td>
<td>260 720</td>
</tr>
<tr>
<td>HC</td>
<td>50</td>
<td>25</td>
<td>0.684 880</td>
<td>354 990</td>
</tr>
<tr>
<td>DT</td>
<td>50</td>
<td>25</td>
<td>0.228 290</td>
<td>1506 4200</td>
</tr>
</tbody>
</table>

DT. Adaptation to PQ** could be demonstrated with DT when the cells were incubated in the 50 μM PQ**-containing medium for 24 h prior to dilution with fresh medium (data not shown).

Adaptation to PQ**—Superoxide dismutase is only one of a team of defensive enzymes. Optimal adaptation to the oxidative stress imposed by PQ** should involve balanced induction of these defensive enzymes and of repressible enzymes as well. It appeared possible that gross overproduction of manganese-containing superoxide dismutase in DT would actually prevent induction of other enzymes both because of competition for the amino acids, metal prosthetic groups, or even the ATP needed to drive protein biosynthesis and because of depression of [O2]. Since glucose-6-phosphate dehydrogenase is one of the proteins whose synthesis is controlled by the O2-inducible, or sox operon, we examined its induction by PQ** in both the HC and DT strains. Table I demonstrates that the HC strain markedly increased its content of active glucose-6-phosphate dehydrogenase when exposed to PQ**, whereas the DT strain did so to a much lesser degree. Induction of superoxide dismutase was also explored (Table I). In the absence of PQ**, DT contained ~2.5 times more active superoxide dismutase than did HC. PQ** caused approximately a 3-fold increase in superoxide dismutase activity in both strains. At high rates of biosynthesis of the manganese-containing superoxide dismutase polypeptide, the metal prosthetic group could become limiting. This was explored by enriching the medium with 25 μM Mn(II), in which case DT exposed to PQ** exhibited a superoxide dismutase-specific activity of 1506 units/mg protein! Since the pure manganese-containing superoxide dismutase has a specific activity of 4500 units/mg, it is clear that 33% of the extractable protein was then manganese-containing superoxide dismutase. It is easy to understand how such single-minded devotion of the protein-synthesizing machinery of the cell to the production of manganese-containing superoxide dismutase would prevent induction of other proteins, such as glucose-6-phosphate dehydrogenase. It should be noted that addition of Mn(II) to the medium did not affect induction of glucose-6-phosphate dehydrogenase.

Discussion

We have confirmed that gross overproduction of manganese-containing superoxide dismutase, by virtue of a multicopy plasmid bearing the sodA gene, increases the sensitivity of E. coli to paraquat (11). We have shown that this reflects an increased susceptibility to the growth-inhibiting effect of paraquat and not to its killing effect. The control strain adapts to paraquat and thus gradually escapes from its growth-inhibiting effect, whereas the manganese-containing superoxide dismutase-overproducing strain is relatively defective in this adaptation.

O2 induces a constellation of enzymes in E. coli that constitute the soxR region (13, 14). It appeared likely that gross overproduction of manganese-containing superoxide dismutase would suppress induction of the other enzymes of this regulon both because it lowers the steady level of O2 and because of simple competition for amino acids and ATP. Glucose-6-phosphate dehydrogenase, which is a member of the soxR regulon, was chosen as a case in point, and its accumulation in response to paraquat was indeed much greater in the control than it was in the manganese-containing superoxide dismutase-overproducing strain. Glucose-6-phosphate dehydrogenase supplies the NADPH, which is needed for the actions of alkylhydroperoxide reductase (20) and glutathione reductase (21) which is thus germane to counteracting the toxic effects of paraquat. A deficit in glucose-6-phosphate dehydrogenase and, therefore, in NADPH and GSH could well compromise the ability of cells to grow in the presence of dioxygen plus paraquat. Greenberg et al. (13) have reported that mutants in glucose-6-phosphate dehydrogenase are indeed hypersensitive towards H2O2 and to menadione.

In addition to glucose-6-phosphate dehydrogenase, the soxR regulon includes endonuclease IV and approximately six other proteins of currently unknown functions. Chan and Weiss (22) have reported that endonuclease IV was more readily induced by paraquat in an E. coli mutant lacking superoxide dismutase than in a control strain. In this case, allowing the [O2] to rise because of a lack of superoxide dismutase increased expression of this member of the soxR regulon. It appears likely that suppression of [O2] by overproduction of superoxide dismutase would adversely affect control of the entire regulon and thus compromise adaptation to paraquat in a variety of ways.

References

8. Bermingham-McDonogh, O., Gralla, E. B., and Valentine, J. S.
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