Superoxide Dismutase Protects against Aerobic Heat Shock in Escherichia coli

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Exposure of a superoxide dismutase-null (sodA sodB) strain of Escherichia coli to aerobic heat stress (45 to 48°C) caused a profound loss of viability, whereas the same heat stress applied anaerobically had a negligible effect. A superoxide dismutase-competent parental strain was resistant to the lethal effect of the aerobic heating. It follows that aerobic heating imposes an oxidative burden of which O$_2^-$ must be a major component. This effect is not seen at 53°C, presumably because, at this higher temperature, direct thermolability of vital cell components overrides the effect of superoxide radicals.

There are data supporting the view that aerobic heat shock imposes an oxidative stress and that oxidative stress can induce a heat shock response both in prokaryotes and in eukaryotes. Thus, menadione caused a heat shock response in Drosophila spp. (9), and H$_2$O$_2$ applied to Drosophila polytene nuclei did likewise (4). Depletion of GSH in cultured cells, with transition metals or with diimide, also induced a heat shock response (10). H$_2$O$_2$ elicits heat shock proteins in Escherichia coli (8), and exposure to 48°C caused induction of Mn superoxide dismutase (MnSOD) in E. coli (15). This induction appeared secondary to increased O$_2^-$ production at the elevated temperature, because it was dioxygen dependent and because cyanide-resistant respiration was elevated at higher temperatures. An increase in cyanide-resistant respiration with elevation of temperature and concomitant induction of SOD were also reported for rat lung (5). Induction of SOD was also caused by heating in Halobacterium halobium (2) and Porphyromonas gingivalis (1).

All of these results can be explained on the basis of an increase in production of O$_2^-$, and consequently of H$_2$O$_2$, at elevated temperatures, with induction of SOD as an adaptive response. This leads to the expectation that a SOD-null strain of E. coli should be more sensitive to heat killing than a parental strain. Yet Kogoma and Yura reported there was no difference between SOD$^+$ and SOD$^-$ strains in sensitivity to 53°C (7). We have reexamined the heat lability of SOD$^+$ and SOD$^-$ strains and find that the lack of SOD markedly sensitizes E. coli to heat stress. Because it is now known that E. coli contains a Cu,ZnSOD, in addition to the MnSOD and FeSOD (3), we also examined the effect of heat on this Cu,ZnSOD.

Bacteria. E. coli J132 was the sodA sodB strain, and AB1157 was the corresponding parental strain (6). Cells were grown in Luria-Bertani medium (10 g of Bacto-Tryptone per liter, 5 g of yeast extract per liter, 10 g of NaCl per liter) adjusted to pH 7.4 with K$_2$HPO$_4$. When antibiotic effects were being examined, the Luria-Bertani medium was replaced with a medium containing M9 medium (13) plus 150 mg of pantothenate per liter, 50 mg of thiamine per liter, and 0.2% Casamino Acids (pH 7.4). Cultures were grown aerobically at 37°C for 16 to 20 h, and aliquots of these overnight cultures were then exposed to the specified higher temperatures either aerobically in a water bath shaker or anaerobically in a BBL Gas Pack system. Temperatures were measured directly in the cell suspensions. For cultures in the Gas Pack, it was necessary to have water in the anaerobic chamber as a heat conductor from the thermostatted bath to the cultures. At intervals, aliquots were taken for dilution, plating, and enumeration after 24 and 48 h at 37°C.

Enzyme assays. Cells were washed once with 50 mM phosphate buffer (pH 7.8) and were then extracted by osmotic method (14), while protein was assayed colorimetrically (12).

Results. (i) Heat kill. The sodA sodB strain suffered a 6-log loss of viability in 4 h at 45°C and a comparable loss in 2 h at 48°C under aerobic conditions. In contrast, there was negligible heat killing under anaerobic conditions, even at 48°C. The exacerbation of thermal sensitivity by air suggested a contributory role for oxidative stress, and the much greater resistance of the SOD-competent strain establishes that O$_2^-$ was a major factor in the aerobic sensitization to heat killing. Figure 1 presents the results of a representative experiment which was repeated five times with little variability.

Because Kogoma and Yura (7) failed to see an effect of SOD on the rate of loss of viability of E. coli exposed to 53°C, we
repeated their conditions. As shown in Fig. 2, the differential thermal sensitivity of the sodA sodB and parental strains, which was so pronounced at 43 and 48°C (Fig. 1), disappeared at 53°C.

(ii) Cu,ZnSOD. We have previously noted that the periplasmic Cu,ZnSOD of E. coli is induced by aerobic conditions (3). It appeared likely that it might be induced by increased O₂⁻ production at elevated temperatures. When a mid-log-phase culture of the sodA sodB strain was incubated at 43°C, there was a moderate increase in Cu,ZnSOD over 3 h, followed by a steep decline. At 48°C only a loss was seen. These results are shown in Fig. 3. Similar results were obtained with early-stationary-phase cultures (results not shown). The Cu,ZnSOD of E. coli is thermolabile, and incubation of an extract of sodA sodB cells at 43°C occasioned a complete loss of SOD activity in 2 h, as shown in Fig. 4, although it was stable at 37°C. The increase in biosynthesis of the Cu,ZnSOD at 43°C must therefore have been more pronounced than is shown in Fig. 3, because it was counterbalanced by a loss of activity due to the instability of this enzyme at this temperature. That this was the case was shown by inhibiting protein synthesis with tetracycline. When this was done, the moderate increase in Cu,ZnSOD activity otherwise seen at 43°C was replaced by a sharp decline (Fig. 3, line 2). Agents (other than heat) that have also been reported to elicit a heat shock response, such as ethanol (8) and nalidixic acid (11), did not cause induction of Cu,ZnSOD (data not shown).

Discussion. The absence of MnSOD and FeSOD imposes a heightened sensitivity to elevated temperatures (43 to 48°C) in E. coli, and this heat killing was oxygen dependent. These results are best accommodated by postulating increased production of O₂⁻ at elevated temperatures and a major role for that O₂⁻ in causing the loss of viability. Kogoma and Yura (7) came to a different conclusion, because they chose to examine loss of viability at a higher temperature (53°C). We could repeat their results at this temperature. It appears likely that direct thermal lability of vital cell components at this higher temperature caused the loss of viability and masked the contribution of O₂ radicals, which could be demonstrated at 43 to 48°C.

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