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Communication

Role of *rpoS* in the regulation of glyoxalase III in *Escherichia coli*✪

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Methylglyoxal is an endogenous electrophile produced in *Escherichia coli* by the enzyme methylglyoxal synthase to limit the accumulation of phosphorylated sugars. In enteric bacteria methylglyoxal is detoxified by the glutathione-dependent glyoxalase I/II system, by glyoxalase III, and by aldehyde reductase and alcohol dehydrogenase. Here we demonstrate that glyoxalase III is a stationary-phase enzyme. Its activity reached a maximum at the entry into the stationary phase and remained high for at least 20 h. An *rpoS*$^-$ mutant displayed normal glyoxalase I and II activities but was unable to induce glyoxalase III in stationary phase. It thus appears that glyoxalase III is regulated by *rpoS* and might be important for survival of non-growing *E. coli* cultures.

Methylglyoxal is a highly toxic electrophile which can be formed non-enzymatically from trioses under physiological conditions (Phillips & Thornalley, 1993; Richard, 1993). In *Escherichia coli* it is produced enzymatically to relieve the inhibition that would occur if sugar phosphates accumulate (Ferguson *et al.*, 1998b; MacLean *et al.*, 1998). In enteric bacteria methylglyoxal is detoxified by three major routes: 1) glutathione-dependent glyoxalase I/II system (Ferguson *et al.*, 1998b); 2) glutathione-independent glyoxalase III (Misra *et al.*, 1995); and 3) NADPH-dependent aldehyde reductase and NADH-dependent alcohol dehydrogenase. Among these three, the GSH-dependent glyoxalase I/II system was reported to be the most important (MacLean *et al.*, 1998). On the other hand,

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Abbreviations: LB, Luria–Bertani medium.

glyoxalase III was found to be more active than glyoxalases I and II (Misra *et al.*, 1995), but the physiological role of this enzyme is not known. Here we demonstrate that the activity of glyoxalase III is growth-dependent, reaching a maximum in the stationary phase, and that this enzyme is regulated by *rpoS*. This finding suggests that glyoxylase III might be important for the survival of non-growing bacterial cultures.

MATERIALS AND METHODS

Strains and media. The strains of *E. coli* used were as follows: ZK126 = parental, and ZK1000 *rpoS*::kan (provided by Dr. J. Imlay, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.). Cultures were grown in LB or M9CA medium. LB medium contained 10 g Bacto-tryptone, 5 g yeast extract, and 10 g NaCl per liter and was adjusted to pH 7.0 with about 1.5 g of K_2HPO_4. M9CA medium consisted of minimal A salts (Maniatis *et al.*, 1982), 0.2% casamino acids, 0.2 % glucose, 3 mg pantothenate, and 5 mg of thiamine per liter.

Growth conditions. Strains were grown overnight at 37°C, with shaking in air, in LB medium containing 50 μg/ml kanamycin where required. Overnight LB cultures were diluted 200-fold in LB medium and were grown to A_{600} about 0.8. Since media affect glyoxalase I and III activities (Misra *et al.*, 1995), mid-log culture was diluted 20-fold into fresh LB or M9CA medium and effect of growth on the glyoxalase activities was monitored. When needed, freshly prepared filter-sterilized methylglyoxal solution was added.

Growth was monitored by measuring the absorbance at 600 nm.

Assays. Cells were harvested, washed three times in 50 mM potassium phosphate, pH 7.5, resuspended in the same buffer and lysed in a French press. The extracts were clarified by centrifugation and then assayed for glyoxalase I (Oray & Norton, 1982a), glyoxalase II (Oray & Norton, 1982b), and glyoxalase III (Misra *et al.*, 1995). Enzyme activities are expressed as units/mg cell protein. A molar absorption coefficient of 3.37×10^{-3} M^{-1} cm^{-1} for *S*-D-lactoylglutathione was used. One unit is defined as the amount of enzyme catalyzing the formation (glyoxalase I) or utilization (glyoxalase II) of 1 μmol/min *S*-D-lactoylglutathione. One unit of glyoxalase III is defined as the amount of enzyme required to utilize 1 μmol/min of methylglyoxal.

RESULTS

Figure 1 demonstrates the relationship between growth in LB medium (Panel A) and

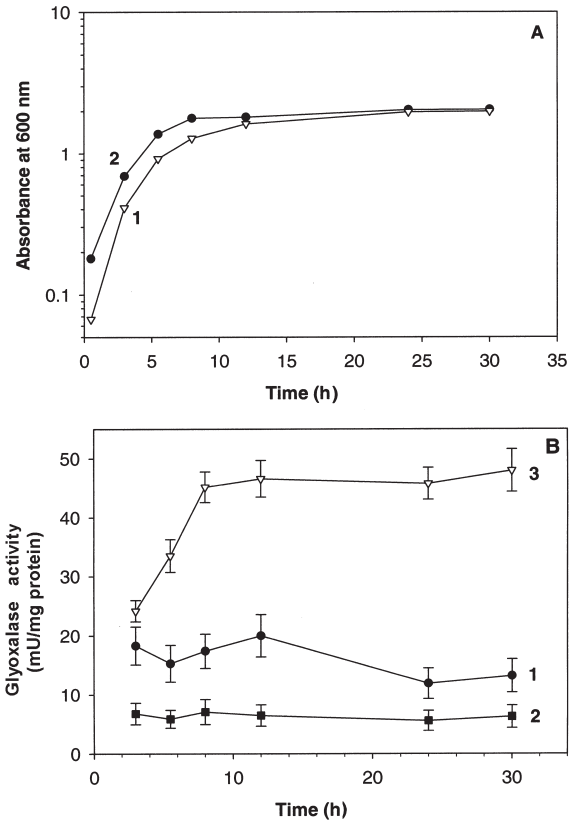

Figure 1. Glyoxalase activities during growth of *E. coli*.

Cells were grown in LB medium as described in Materials and Methods. Panel A, growth: curve 1, with 0.2 mM methylglyoxal; curve 2, without methylglyoxal. Panel B, activities of glyoxalases I, II, and III: line 1, glyoxalase I; line 2, glyoxalase II; line 3, glyoxalase III.

the activities of glyoxalases I and III (Panel B). As seen, glyoxalases I and II are constitutive and did not change much during growth. In contrast, glyoxalase III activity depended on the growth phase, reaching a maximum at entry into the stationary phase, and remaining constant for at least 20 h in the stationary phase (Fig. 1, B). These changes in the glyoxalase III activity did not depend on the growth medium and followed a similar pattern in both LB and M9CA media. Addition of methylglyoxal (10–100 μM) affected neither the glyoxalase activities, nor cell growth (not shown). At higher concentrations (200 μM) methylglyoxal slowed down the growth (Fig. 1A) but did not cause induction of any of the glyoxalases.

rpoS is a regulatory gene of central importance for early stationary phase-induced genes in *E. coli* (Hengge-Aronis *et al.*, 1993). As glyoxalase III is maximally induced in the stationary phase, it might be expected that this enzyme is regulated by *rpoS*. Furthermore, no induction occurred after the cells reached the stationary phase of growth (about 8 h), which is when RpoS is at its maximal level (Lange & Hengge-Aronis, 1994). Figure 2 shows that, an *rpoS*$^-$ mutant contained glyoxalase I activity similar to that in the parental, RpoS-competent strain, while glyoxalase III remained very low in the stationary phase.

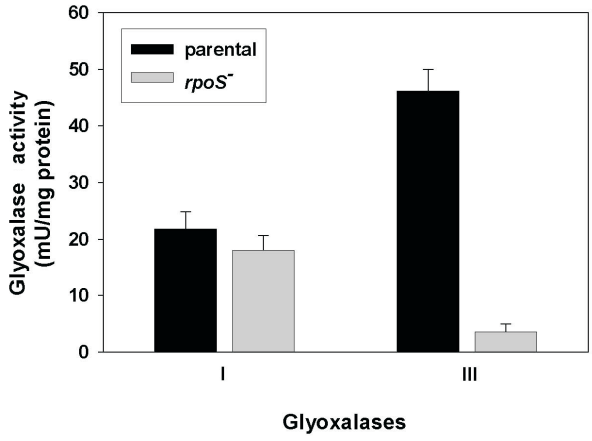

Figure 2. Glyoxalase activities in *rpoS*$^-$ mutants.

Parental and *rpoS*$^-$ cultures were grown in LB medium for 12 h, harvested by centrifugation and assayed for glyoxalases.

DISCUSSION

When *E. coli* cells enter stationary phase due to depletion of nutrients, they become resistant to a wide range of harmful environmental conditions (Hengge-Aronis, 1993). The development of stationary phase resistance and the changes that occur in the stationary phase are brought about by changes in gene expression. Many of these changes depend on the alternative sigma factor σ^S (RpoS). It is required for the induction of more than 30 genes in the stationary phase (Hengge-Aronis, 2000), and many of them provide stationary phase resistance. The genes under control of σ^S are responsible for resistance to high temperature, ethanol, osmotic shock and oxidizing agents (Hengge-Aronis, 1993) and some are involved in DNA repair and protection (Almiron *et al.*, 1992). Thus, the RpoS-regulated genes play a central role in stationary phase resistance to harmful environmental conditions.

Bacteria are exposed to methylglyoxal that is either endogenously produced or comes from the environment. Since methylglyoxal is cytotoxic, survival of cells requires protective mechanisms. In exponentially growing cells glutathione is the most important of these protective mechanisms (Ferguson & Booth, 1998; MacLean *et al.*, 1998). Stationary phase cells are more resistant to methylglyoxal and other electrophiles (Ferguson *et al.*, 1998a), and there are various factors known so far that contribute to this resistance. Among them are: *i*, an increase of GSH level in stationary phase *E. coli* (Loewen, 1979), due to the RpoS-dependent induction of glutathione reductase (gor) (Becker-Hapak & Eisenstark, 1995), *ii*, activation of the KefB and KefC potassium channels (Ferguson *et al.*, 1998a) and *iii*, induction of the RpoS-dependent exonuclease III (Smirnova *et al.*,

1994). Here we demonstrate that the activity of a methylglyoxal-metabolizing enzyme, glyoxalase III, is increased in the stationary phase and the increase is RpoS-dependent. It appears therefore that glyoxalase III might be important for the survival of non-growing *E. coli* cultures by providing an additional route for detoxification of methylglyoxal and other electrophiles.

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