Short Communication

An Increase in Levels of Glutathione in *Escherichia coli* B caused by Osmotic Stress

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In all living cells, maintenance of turgor pressure is a prerequisite for normal growth and division, and closely connected with the adaptive mechanism that protects cells from the dehydration caused by salt or freezing. Glycinebetaine and amino acids, such as proline, if they are present in the medium, accumulate in *Escherichia coli* cells at high concentrations during stress. Potassium ions are accumulated by bacteria and play a crucial role in the osmoregulation of cells. In our previous paper, we found an increase in the concentration of ATP in *E. coli* as a specific result of osmotic stress.

Glutathione is a tripeptide present in all living cells and has been reported to have a palliative effect under various stressful conditions. It appears to be involved in thermotolerance, radioprotection, the induction of stress protein, and so on. Until now, there have been no studies, to our knowledge, of the role of glutathione in osmoregulation. In this report, we describe our studies on the levels of glutathione in *E. coli* B cells under osmotic stress.

*E. coli* B cells were grown aerobically in glucose mineral medium at 37°C. Growth was monitored by measurement of the optical density of the culture at 600 nm. An optical density at 600 nm of 0.3 units was equivalent to a cell density of 0.169 mg dry weight per ml. Cells were stressed by the addition of a concentrated solution of NaCl or sucrose to the culture when the cells were in the logarithmic phase of growth (OD$_{600}$ of about 0.3). An appropriate volume of culture was filtered under aspiration over a membrane filter and extracts of the cells were prepared by the methods described previously. Levels of glutathione present in the extracts were measured by an enzymatic recycling procedure.

In unstressed cells, levels of reduced glutathione (GSH) increased gradually from the early to the late logarithmic phase of growth and then the level decreased gradually. The concentration of oxidized glutathione (GSSG) was less than 0.1 nmol per mg dry weight of cells at all phases of growth (data not shown). The changes in levels of glutathione in cells after treatment with sucrose (final concentration, 0.51 M) or NaCl (final concentration, 0.5 M) were measured as described in the text. Levels of glutathione (sum of reduced and oxidized forms) are expressed in terms of the oxidized form per mg dry weight of cells. More than 95% of the glutathione was in the reduced form. (A) Growth curves: control, (○); plus NaCl, (●); plus sucrose, (▲). (B) Glutathione: control, (★); plus NaCl, (●); plus sucrose, (△).
Levels of GSH in the cells increased to about 2.5 (the addition of sucrose) and 4 fold (the addition of NaCl) that in the control cells in the first 15 and 30 min, respectively (Fig. 1B). These results suggested that the increase in levels of GSH in the cells might be caused by the osmotic stress. The elevated levels of GSH showed a tendency to decrease with the resumption of growth (Fig. 1B). During this period, levels of GSSG increased only slightly, to below 0.4 nmol per mg dry weight cell.

The increased level of GSH was dependent on the concentration of NaCl. Figure 2 shows the relative levels of GSH in cells harvested 10 min after treatment with various concentrations of NaCl. The level of GSH in cells increased in proportion to the final concentration of NaCl, which was between 0.1 and 0.4 M (Fig. 2). When the external concentration of NaCl exceeded 0.6 M, GSH in the cells was excreted into the medium (data not shown).

Previously, we reported that after the addition of NaCl, the concentration of K+ in the cells increased immediately and that K+ is a principal solute in osmoregulation among other solutes such as proline and betaine. The finding that the GSH level in the cells rose several-fold in an early stage of the addition of NaCl suggests that GSH might be contributed to K+ transport under the condition of osmotic stress. Meury and Kepes reported that GSH is important in the gating process related to the two efflux systems that are associated with K+ channels, Trk B and Trk C.

Recently, a peptide that accumulates during osmotic stress was found, suggesting that peptides contribute to osmoregulation. It is known that GSH acts in the detoxication of a number of drugs and that administration of these drugs increases levels of GSH. Our preliminary studies showed that chloramphenicol stimulated the synthesis of GSH and that almost all of the increased amounts of GSH effluxed into medium. However, the stimulation of the synthesis of GSH ceased immediately upon the addition of NaCl (T. Ohwada and S. Sagisaka, manuscript submitted), indicating that different mechanisms for the stimulation of the synthesis of GSH might be operative within the cell.

References