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Topoisomerase Activity during the Heat Shock Response in *Escherichia coli* K-12

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During the upshift of temperature from 30 to 42, 45, 47, or 50°C, an increase in the level of supercoiling of a reporter plasmid was observed. This increase was present in *groE* and *dnaK* mutants but was inhibited in cells treated with chloramphenicol and novobiocin. The intracellular [ATP]/[ADP] ratio increased rapidly after an upshift in temperature from 30 to 47°C and then decreased to reach a level above that observed at 30°C. These results suggest that gyrase and proteins synthesized during heat shock are responsible for the changes seen in plasmid supercoiling. Proteins GroE and DnaK are probably not involved in this phenomenon.

Escherichia coli DNA is organized into independently negative supercoiled loops (25, 31). In vivo, the level of DNA supercoiling is regulated mainly by a balance between the opposing activities of topoisomerases I and II. Topoisomerase I relaxes negatively supercoiled DNA, while topoisomerase II (gyrase) uses ATP to increase negative supercoiling (23, 28). The [ATP]/[ADP] ratio strongly influences the level of plasmid supercoiling reached in vitro with purified gyrase (29). It has also been shown that an increase in the intracellular [ATP]/[ADP] ratio correlates with an increase in plasmid supercoiling (13, 14). These data suggest that the [ATP]/[ADP] ratio plays an important role in the control of DNA supercoiling. Replication, transcription, and DNA-binding proteins can also modify the level of DNA supercoiling (7). Regulation of DNA supercoiling is a homeostatic process essential to the maintenance of normal cell functions (18, 21, 27). It is well established that expression of a number of genes is influenced by the level of DNA supercoiling (1, 5, 6, 11–14, 26).

In vitro, temperature changes alter the DNA helical pitch and, thus, alter the level of supercoiling. An increase in temperature induces a decrease in the helical pitch and a lower level of DNA supercoiling (4, 22). However, physiological experiments with a small plasmid as a reporter of DNA supercoiling showed that plasmids isolated from cells grown at 37°C were more highly supercoiled than those from cells grown at 17°C (10). At 10 to 15 min after cultures had been shifted from 17 to 37°C, the plasmid topoisomer distribution reached the distribution of plasmid molecules extracted from cells grown for many generations at 37°C. The interpretation of these results was that a temperature upshift decreases intracellular DNA supercoiling and that gyrase is temporarily favored until supercoiling regains its preshift level. However, experiments to show that gyrase was involved were not presented (10).

The purpose of the present work was to extend these studies to cells exposed to higher temperatures. The cellular ability to change the level of plasmid DNA supercoiling was analyzed in cells grown at 30°C and then upshifted to 42, 45, 47, or 50°C. This ability was determined indirectly by using a small pBR322-derived plasmid as a reporter and with temperatures usually used to induce the heat shock response. Plasmid topoisomer distribution must mainly reflect the activity of the topoisomerases present in the cell. The study of plasmid supercoiling in cells exposed to high temperatures is particularly interesting, since purified gyrase is 50% inactivated by incubation at 46 or 55°C for 5 min (8, 20). After 10 min of incubation at 46°C, only approximately 35% of the gyrase activity remained (20).

To determine the variation of plasmid DNA supercoiling during the upshift of temperature from 30 to 42, 45, 47, or 50°C, the level of supercoiling of a plasmid was measured at different times during the heat treatments. Plasmid pMS01, a tetracycline-sensitive ampicillin resistance derivative of pBR322 (16), was used as a reporter plasmid. Plasmid pMS01 topoisomers, which were isolated by the alkaline lysis method (24) from W3110 cells that were exposed to high temperatures, were separated by electrophoresis in 1% agarose horizontal gels in the presence of chloroquine (10 μg/ml). Under these experimental conditions, topoisomers that were more supercoiled before electrophoresis migrated faster through the gel. The results obtained when cells were exposed to 47°C are presented in Fig. 1. A slight increase in the level of plasmid supercoiling was detected as early as 5 min after exposure to this temperature (Fig. 1, lanes a and b); this level increased with time, reaching a maximum at 15 min (Fig. 1, lane d). After this period, the level of plasmid supercoiling decreased slightly and remained practically unchanged (Fig. 1, lanes e and f). Changes in the level of plasmid supercoiling were less pronounced when cells were exposed to 42°C than when they were exposed to 45, 47, or 50°C. Cell exposure to 45, 47, and 50°C induced a similar increase in the level of plasmid supercoiling (data not shown). Similar results were obtained with cells grown in Luria broth (19) or minimal medium (19) and with the following different *E. coli* strains: W3110 prototroph, MC1061 prototroph, C600 (*thr leu thi*), and AB1157 (*thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 argE3 rpsL31 tsx-33 supE44 his-4 recE*) (data not shown). These results showed that upshifts to high temperatures, even to those at which gyrase is probably not fully active, induced an increase in the level of plasmid supercoiling.

To determine whether the observed increase in plasmid su-

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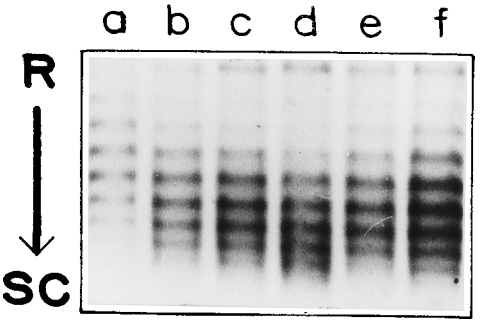

FIG. 1. Distribution of plasmid pMS01 topoisomers isolated from cells grown in Luria broth (19) after a temperature upshift from 30 to 47°C. Cultures of W3110 cells growing at 30°C were shifted to 47°C. Plasmid DNA was isolated and electrophoresed in agarose gels containing 10 μg of chloroquine per ml. The samples were electrophoresed for 19 h at 2.5 V/cm in 90 mM Tris–10 mM EDTA–90 mM boric acid (pH 8.3). Lanes: a, control cells at 30°C; b through f, topoisomers from cells treated at 47°C for 5, 10, 15, 20, and 40 min, respectively. R, relaxed DNA; SC, supercoiled DNA.

percoiling was catalyzed by gyrase, novobiocin, a specific gyrase inhibitor (9), was used. Bacterial cells in mid-log phase were shifted to 50°C in the presence of novobiocin (80 μg/ml) for 10 min. This concentration of novobiocin had little effect on growth rates and on plasmid supercoiling in cells growing at 30°C (data not shown). Novobiocin inhibited the increase in the level of plasmid supercoiling that was observed when control cells were exposed to 50°C (Fig. 2). Similar results were obtained with upshifts to 47°C (data not shown). This result suggests that gyrase could be involved in the heat-induced increase of plasmid supercoiling.

The possible role of proteins synthesized during the heat shock response in the changes in plasmid supercoiling was studied by using chloramphenicol. When chloramphenicol was added at the time of the temperature shift, it inhibited the increase in the level of DNA supercoiling that was induced by 10 min of exposure at 47°C (Fig. 3, lane d). However, when chloramphenicol was added 5 min after the upshift, it caused only a partial inhibition of the heat-induced increase in plasmid supercoiling (Fig. 3, lane e). These results suggested that chloramphenicol inhibited the translation of a protein(s) synthesized during the first 5 min of the heat shock treatment

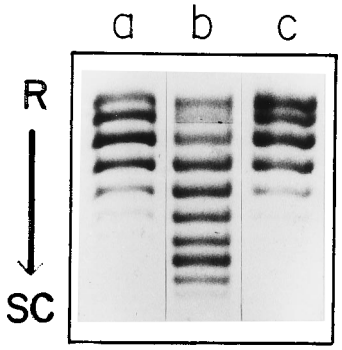

FIG. 2. Effect of novobiocin on the distribution of pMS01 topoisomers isolated from cells grown in Luria broth (19) after a temperature upshift from 30 to 50°C. Plasmid DNA was isolated, and topoisomers were separated on an agarose gel containing 10 μg of chloroquine per ml. The samples were electrophoresed for 19 h at 2.5 V/cm in 90 mM Tris–10 mM EDTA–90 mM boric acid (pH 8.3). Lanes: a, control cells at 30°C; b, cells exposed at 50°C for 10 min; c, cells exposed at 50°C for 10 min in the presence of 80 μg of novobiocin per ml. R, relaxed DNA; SC, supercoiled DNA.

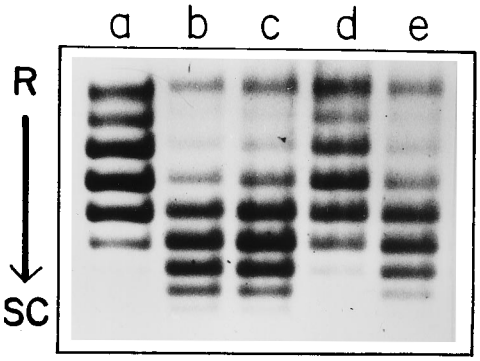

FIG. 3. Effect of chloramphenicol and rifampin on the distribution of pMS01 topoisomers isolated from cells grown in Luria broth (19) after a temperature upshift from 30 to 47°C. Plasmid DNA was isolated, and topoisomers were separated on an agarose gel containing 10 μg of chloroquine per ml. The samples were electrophoresed for 19 h at 2.5 V/cm in 90 mM Tris–10 mM EDTA–90 mM boric acid (pH 8.3). Lanes: a, control cells at 30°C; b, cells exposed at 47°C for 10 min; c, cells exposed at 47°C for 10 min (rifampin [100 μg/ml] was added 5 min before the upshift of temperature); d, cells exposed at 47°C for 10 min in the presence of 50 μg of chloramphenicol per ml; e, cells exposed at 47°C for 5 min and for 5 additional min in the presence of 50 μg of chloramphenicol per ml. R, relaxed DNA; SC, supercoiled DNA.

which is needed to induce this increase in the level of plasmid supercoiling. To evaluate the possible effect of transcription on plasmid supercoiling, rifampin was added 5 min before the upshift. The change in the level of plasmid pMS01 supercoiling that was induced by heat was the same in cells with or without rifampin (Fig. 3, lanes b and c).

Since gyrase is probably not fully active at high temperatures and does not seem to be a heat shock protein (3), one possible explanation for the observed increase in plasmid supercoiling is that the main chaperonines, GroE and DnaK, maintain the gyrase activity at high temperature. However, the thermally induced increase of plasmid supercoiling was similar in the wild type and in CAG9269 (*groEL40*), CAG9259 (*groES30*), CAG13093 (*dnaK756*), CAG13128 (*dnaK302*), and CAG13350 (*dnaK204*) cells (30) (data not shown). Although mutations in *groE* and *dnaK* do not abolish all functions of the proteins encoded by these genes, results obtained with cells mutated in these genes suggest that both chaperonines do not play an important role in the thermally induced increase of plasmid supercoiling. To further examine the role of the chaperonine GroE in the thermally induced increase of plasmid supercoiling, this phenomenon in cells carrying plasmid pLN44 (*groEL groES*) which overproduce GroEL-GroES was studied. Plasmid pACY184 (Cm^r) (2) was used instead of pMS01, since plasmid pLN44, which contains the *groE* operon, encodes ampicillin resistance. Spectinomycin (400 μg/ml) instead of chloramphenicol was used to inhibit protein synthesis. Overproduction of the GroE proteins did not reverse the spectinomycin inhibition of the thermally induced increase in the level of plasmid supercoiling (data not shown). These results and those obtained with the *groE* mutants suggest that the chaperonine GroE does not play a role in this phenomenon.

To study if the heat shock response changes cellular energetics, the intracellular [ATP]/[ADP] ratio was determined. This ratio increased rapidly after the upshift of temperature. After 5 and 10 min of cellular exposure to 47°C, the [ATP]/[ADP] ratios were approximately 3.0 and 4.0, respectively, compared with 1.7 for cells grown at 30°C, and then the ratio decreased to a level higher than that observed before the upshift (Fig. 4). These changes in the [ATP]/[ADP] ratio cor-

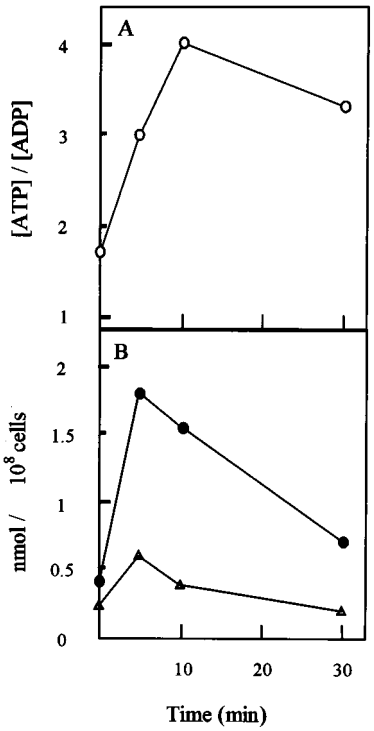

FIG. 4. Cellular [ATP]/[ADP] ratio during an upshift of temperature from 30 to 47°C. For determinations of ATP and ADP concentrations, W3110 cells were grown on minimal medium (19) at 30°C and were shifted to 47°C. At various times, 3 ml of the culture was transferred to tubes containing 0.17 ml of cold 60% (vol/vol) perchloric acid, and the tubes were incubated for 1 h on ice. The samples were neutralized to pH 6.5 to 6.7 with 2.5 M K_2CO_3 and were centrifuged at 6,700 × *g* for 10 min to remove cell debris and the carbonate precipitate. All procedures were performed in the cold room. The cellular ATP and ADP concentrations were determined by HPLC. The samples (100 μl) were loaded directly onto a μ-Bondapak C18 column (125 Å, 10 μm, 3.9 by 300 mm; Waters; Millipore) and were eluted with a solution containing 88% buffer (0.1 M KH_2PO_4–2.5% tetrabutylammonium hydroxide [pH 6.8]) and 12% methanol. The flow rate was 1.6 ml/min. ATP and ADP were detected at 254 nm. The concentrations of ATP and ADP were determined by computer integration of the areas under the corresponding peaks. (A) Cellular [ATP]/[ADP] ratio during exposure of cells to 47°C; (B) cellular concentrations of ATP (●) and ADP (▲) during exposure of cells to 47°C.

relate with the observed heat-induced increase in plasmid supercoiling. The level of plasmid supercoiling increased by approximately two to four topoisomer bands, depending on the temperature upshift (Fig. 1, 2, and 3). This result shows that, like transitions from aerobic to anaerobic growth (13) and to salt shock (14), heat shock increases the cellular [ATP]/[ADP] ratio and the level of DNA supercoiling. The increases in the levels of DNA supercoiling induced by a transition to anaerobic growth and by salt and heat shock are similar; however, the increase in the [ATP]/[ADP] ratio induced by salt shock was higher than the increase in the ratios induced by a transition to anaerobic growth and by heat shock. Changes in this ratio are similar under the last two conditions (13, 14; this work).

The effect of heat shock on the synthesis and/or function of DNA topoisomerases is unclear. The present work shows that upshifts in temperature from 30°C to 42, 45, 47, or 50°C (Fig. 1; data not shown) induce an increase in plasmid supercoiling, as reported for upshifts from 17 to 37°C (10). Therefore, the enzyme(s) responsible for the increase in plasmid supercoiling is active at high or even at lethal temperatures. The inhibition of the heat-induced increase in the level of plasmid supercoiling by novobiocin and the observed increase in the intracellular [ATP]/[ADP] ratio during heat exposure strongly suggest that gyrase is involved in this heat-induced increase in the level of plasmid supercoiling. The results also show that proteins synthesized during heat shock play a role in the changes in the level of plasmid supercoiling. Although the participation of chaperonines in gyrase activity at high temperatures seems unlikely, additional experiments are needed to rule this out.

An increase in the level of *gyrA* and *gyrB* gene expression caused by the heat-induced initial relaxation of DNA could be expected (18). However, the Gyr proteins are not included in the list of approximately 40 heat shock proteins described elsewhere (3). On the contrary, both GyrA and GyrB proteins were recently identified as cold shock proteins (15). The gene *topA*, which encodes topoisomerase I, has one σ^{32} promoter and three σ^{70} promoters (17). However, despite changes in the activities of these promoters during heat shock, the amount of topoisomerase I remained relatively constant. The level of topoisomerase I activity at high temperature was not determined (17).

To improve the understanding of the mechanism that links heat shock with topoisomerase activity, it is important to study the transcription of *gyrA* and *gyrB* genes and the cellular concentration of gyrase during the heat shock response, as well as the levels of topoisomerase I and II activities at different temperatures in the presence and absence of the chaperonines GroE and DnaK.

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