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Heat Damage and Repair in the *Escherichia coli* Nucleoid: Kinetics Based on Sedimentation Analysis

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Changes in the structure of the *Escherichia coli* nucleoid during heat damage and repair were followed by sedimentation analysis in neutral sucrose gradients. Heating at 50° C results first in a decrease in the sedimentation coeficient of the isolated nucleoid. Increasing the heating time, a subsequent increase in sedimentation coefficient is observed.

After a heat shock (i.e. 4 min at 50° C), a short incubation at 25° C (i.e. 5 min) allows the nucleoid to repair and return to the sedimentation coefficient of control unheated nucleoids. The nucleoids heated at 50° C for longer periods and incubated afterwards at 25° C demonstrate a different pattern of structural repair. They associate with protein in the first stage of the repair period.

Elucidation of the mechanisms of heat damage and repair has a wide range of applications from cancer therapy to food safety. In cancer research, thermal treatments (hyperthermia) have proven useful in cancer therapy (4, 17). In food safety, heat damage and repair is important in the application of thermal processing to destroy pathogenic or saprophytic microorganisms in food products.

The *Escherichia coli* nucleoid is composed of folded and supercoiled DNA organized with RNA and protein in an structure having dimensions, superhelical density, and number of DNA domains similar to the chromosome *in vivo* (8, 11, 15). The maintenance of the DNA supercoiling is important because it seems to play a role in every DNA-related process (1, 2, 5, 16). The *E. coli* nucleoid has been shown to be a useful system to study heat damage and repair (7, 9). PELLÓN and GÓMEZ (7) have reported that the recovery of the native nucleoid structure appears to be required for the cell to reinitiate growth and division after heat damage.

The present study reports on the kinetics of structural changes that the *E. coli*

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nucleoid undergoes after heat treatments at lethal temperatures (50° C).

Materials and Methods

Cell labeling and nucleoid isolation. The basic experimental procedures have been previously described (8, 10). The following minor changes were carried out in order to rapidly isolate the nucleoids after heating. *E. coli* K-12 KU105 was inoculated into a defined medium with ^{14}C-thymidine or with ^{14}C-thymidine and ^{3}H-leucine and the cells labeled during growth at 37° C (8, 10). At early exponential phase, the cells were harvested by centrifugation and the cell pellet was resuspended in the same defined growth medium without label. Aliquots of 0.1 ml (4×10^8 cells) were transferred, at 25° C, to 12 × 75 mm glass tubes. The tubes were heated at 50° C for various times, followed by the addition of 0.1 ml of double strength solution A at 0° C.

Solution A contained 20% (wt/vol) RNase-free sucrose, 0.1 M NaCl, and 0.01 M tris-hydrochloride buffer (pH 8.2). The remaining procedure for the nucleoid isolation by spheroplast formation and lysis was carried out by the method described by PELLÓN *et al.* (8, 10). The cell lysates containing the nucleoids were sedimented in 10-50% neutral sucrose gradients at 4° C and 4,000 rpm for a total $\omega^2 t$ of 6×10^9 rad^2/s. The gradients were fractionated from the top.

Results and Discussion

When heating *Escherichia coli* cells at lethal temperatures (50° C), the sedimentation coefficient (Sn) of their isolated nucleoids changes as a function of the heating time (Fig 1). Nucleoids isolated from control unheated cells have a Sn of 1800-1900 S (Fig. 1A). The Sn of nucleoids isolated from heat-shocked cells (i.e. 2-4 min at 50° C) decreased from 1800 S to approximately 1200 S (Fig. 1A, B). This decrease in Sn could reflect structural alterations in the nucleoid due to either a loss of DNA supercoiling or an unfolding

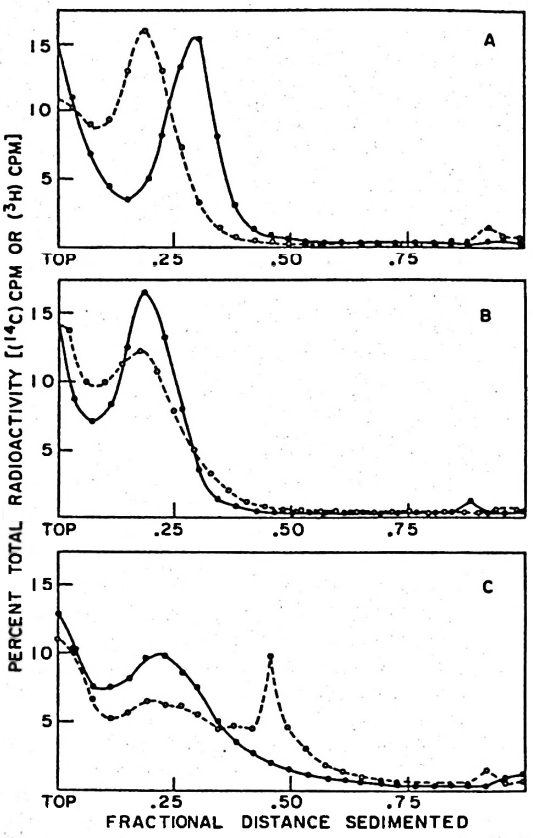

Fig. 1. *Sedimentation profiles for nucleoids from heated cells.*
The experimental procedure is indicated in Materials and Methods. The cell cultures were labeled during growth at 37° C with ^{14}C-thymidine. After the heating at 50° C, the cells were cooled to 0° C immediately after heat treatment.

A. ●——● Unheated control (^{14}C= 16,000 cpm)
O——O 50° C 2 min (^{14}C= 16,000 cpm)

B. ●——● 50° C, 4 min (^{14}C= 19,000 cpm)
O——O 50° C, 6 min (^{14}C= 17,000 cpm)

C. ●——● 50° C, 8 min (^{14}C= 17,000 cpm)
O——O 50° C, 10 min (^{14}C= 17,000 cpm)

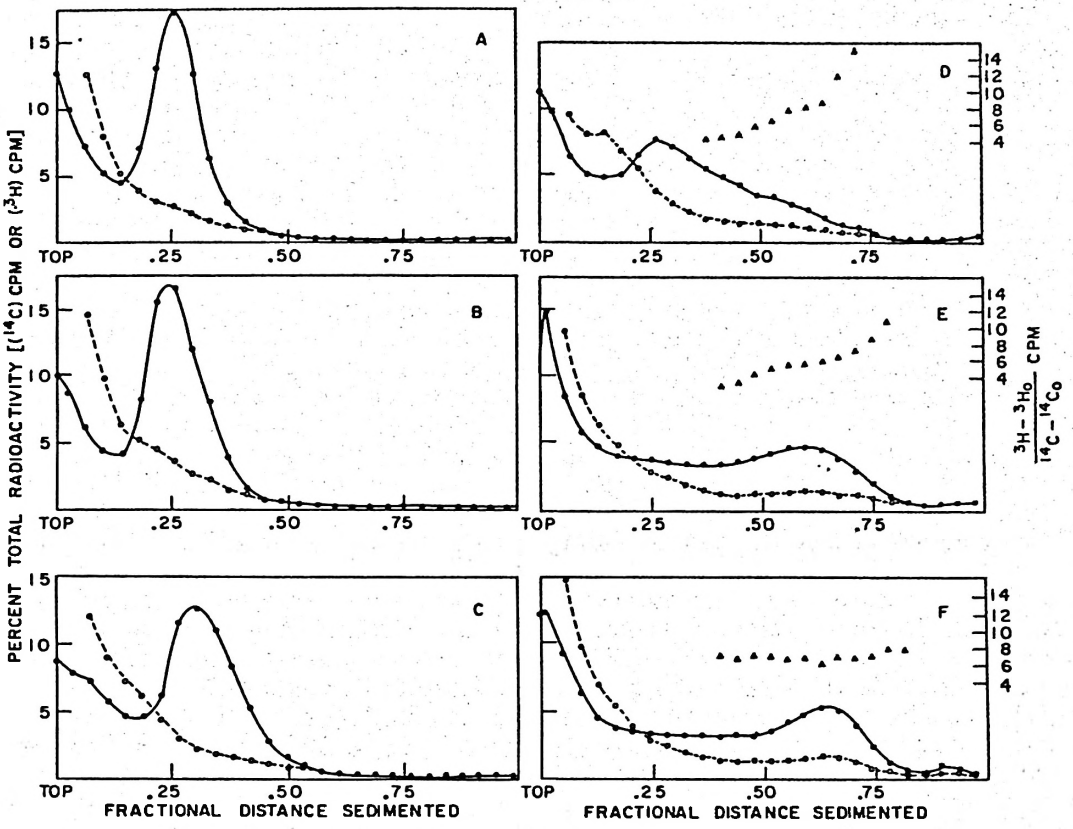

Fig. 2. *Sedimentation profiles for nucleoids from heated cells incubated for 5 min at 25° C after heating* The experimental procedure is indicated in Materials and Methods. The cell cultures were labeled during growth at 37° C with ^{14}C-thymidine (●——●) and ^{3}H-leucine (O——O). After the heating at 50° C, the cells were kept at 25° C for 5 min and then they were lysed to isolate their nucleoids.

A. Control (^{14}C= 17,000 cpm; ^{3}H= 391,000 cpm)
B. 50 ° C, 2 min (^{14}C= 19,000 cpm; ^{3}H= 450,000 cpm)
C. 50° C, 4 min (^{14}C= 18,000 cpm; ^{3}H= 409,000 cpm)
D. 50° C, 6 min (^{14}C= 17,000 cpm; ^{3}H= 415,000 cpm)
E. 50° C, 8 min (^{14}C= 16,000 cpm; ^{3}H= 403,000 cpm)
F. 50° C, 10 min (^{14}C= 15,000 cpm; ^{3}H= 372,000 cpm)

In profiles D, E, and F, the triangles represent the ratios Protein/DNA [(^{3}H - ^{3}Ho) cpm (^{14}C - ^{14}Co) cpm] for the fractions below. The ^{3}Ho and ^{14}Co are the cpm for the same fractions in the control profile (A).

of the structure. Nucleoids isolated from cells heated for longer times (i.e. 8-10 min at 50° C) showed an increase in Sn ranging for approximately 500 to 3300 S, suggesting the condensation of the nucleoid structure.

To investigate the repair process at the nucleoid level, heat treated cells were incubated at 25° C for 5 min and the isolated nucleoids subjected to sucrose gradient analysis. Nucleoids isolated from heat-shocked cells (i.e. 2-4 min at 50° C) allowed to repair for 5 min at 25° C have a Sn of approximately 1800 S, which is the

Sn value of control nucleoids isolated from unheated cells (Fig. 2B, C). These results indicate that heat-shock at 50° C appears to be rapidly repaired at the nucleoid level.

The sedimentation profiles of nucleoids isolated from cells heated at 50° C for longer times and subsequently incubated at 25° C for 5 min showed a wide range in Sn from 1500 S to 4000 S (Fig. 2D-F). In addition, these nucleoids appear now associated with protein (Fig. 2E, F). The formation of fast sedimenting protein structures upon heating at 50° C has been demonstrated by PELLÓN *et al.* (9).

In cells heated at 50° C for 6 and 8 min and allowed to repair for 5 min at 25° C the ratio of ^{3}H-leucine labeled protein to ^{14}C-thymidine labeled DNA for different fractions of the nucleoid sedimentation profile increases as the Sn increases (Fig. 2D-F). On the contrary, for cells heated at 50° C for 10 min and allowed to repair for 5 min, the ratio of protein to DNA in every fraction, does not vary from 3000 to 5000 S. The latter could be the result of two opposite driving forces; (I) the association of the nucleoids with protein (i.e. *de novo* protein structures formed upon heating) (9) that results in an increase in the Sn; versus (II) the unfolding of the nucleoid structure that results in a decrease in the Sn (10). The net result of these two opposite driving forces would be a population of nucleoids with different Sn having the same protein to DNA ratio as in Figure 2F.

Results presented in Figures 1 and 2 indicate that the repair after thermal treatments at lethal temperatures (50° C) shows a different pattern at the nucleoid level depending on the severity of the heat treatment (i.e. heating time). A short heating time (i.e. heat shock) results in a transient change in nucleoid structure which is repaired rapidly and is reflected in a lag time in the growth of the culture without a significant loss in cell viability (6). After more severe heat treatments, the repair process at the nucleoid level includes at least two steps (9). In the first step the nucleoids appear associated with protein (Fig. 2E, F), and in the second step they dissociate the protein and regain the characteristic sedimentation coefficient of nucleoids isolated from unheated cells (7). The dissociation of the nucleoids from the fast sedimenting protein and/or the disappearance of this protein seems to be required for the cell to be able to reinitiate growth and division (7).

The understanding of the mechanisms involved in the repair of the DNA tertiary structure in the nucleoid will require further studies on the nature of the fast sedimenting proteins (9) which associate the nucleoid during repair. In this respect, there is evidence that hyperthermic treatments of eukaryotic cells (i.e. thermal treatments at lethal temperatures, 42-45° C) similar to those employed in tumor therapy, also result in an increase in the amount of protein associated to HeLa cell nucleoids (13) together with alterations in the supercoiling of the DNA. Both prokaryotic and eukaryotic cells are able to dissociate the protein from the DNA after a period of repair at 37° C (7,14). This suggests a general mechanism of response to heat in prokaryotic and eukaryotic cells that should be elucidated to improve the therapeutic value of hyperthermia in the clinical treatment of tumors. The present studies being conducted in *E. coli* should help in establishing the mechanisms by which a cell is either inactivated or able to repair after thermal treatments.

Resumen

Se estudian, mediante sedimentación en gradientes neutros de sacarosa, las alteraciones en la estructura del nucleoide de *Escherichia coli* durante el daño térmico a 50° C y su posterior reparación. El tratamiento térmico a 50° C origina primero una disminución y subsiguientemente un incremento del coeficiente de sedimentación de los nucleoides. Después de un shock térmico (4 minutos a 50° C) la

reparación del nucleoide ocurre en menos de 5 minutos a 25° C. Con tratamientos más prolongados a 50° C la reparación del nucleoide es cualitativamente diferente, ya que durante una primera fase el nucleoide se asocia con proteína.

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