APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1980, p. 358-364
0099-2240/80/00-0358/07$02.00/0

Vol. 40, No. 2

Heat Damage to the Folded Chromosome of *Escherichia coli* K-12†

JOSE R. PELLON, KEVIN M. ULMER,‡ AND REINALDO F. GOMEZ*

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The folded chromosome or nucleoid of *Escherichia coli* was analyzed by low-speed sedimentation in neutral sucrose gradients after in vivo heat treatment. Heat treatment of cultures at 50°C for 15, 30, and 60 min resulted in in vivo association of the nucleoids with cellular protein. Structural changes, determined by the increase in speed dependence of the nucleoids from heated cells, also occurred. These changes were most likely due to the unfolding of the typical compact nucleoid structure. The nucleoids from heated cells also had notably higher sedimentation coefficients (3,000 to 4,500S) than nucleoids from control cells (1,800S). These nucleoids did not contain greater than normal amounts of membrane phospholipids or ribonucleic acid. We propose that the protein associated with the nucleoids from heated cells causes the observed sedimentation coefficient increases.

Thermally injured bacterial cells reportedly suffer changes in most, if not all, of their macromolecular components (1, 5, 11, 19). To determine the cellular events that must occur before a thermally injured cell can resume normal growth, it is necessary to elucidate the structural and functional changes caused by heat.

One of the macromolecular structures that is affected by mild heat treatments is the DNA molecule. Induction of single-strand breaks in the DNA of *Escherichia coli* after heat treatment was demonstrated by Bridges et al. (2, 3). Woodcock and Grigg (23) showed that single- and double-strand breaks are induced in the DNA of *E. coli* by heating the cells, and that the viability of the culture is dependent on DNA repair. Subsequently it was reported (6) that heat treatments in the range of 50 to 60°C were able to induce endonucleolytic breakage in *E. coli*. Enzyme inhibitors, added before heat, substantially reduced the number of DNA breaks, suggesting enzymatic induction by heat.

One of the problems in studying the effects of heat or other damaging agents on specific macromolecular structures such as DNA is that the techniques generally available do not account for the close relationship that exists between macromolecules in vivo. The nucleoid or folded chromosome of *E. coli* (13) is believed to resemble closely the in vivo chromosome (16). It consists of DNA closely organized with RNA and protein in a compact structure (7, 14, 16, 18). Changes in the nucleoid structure are reflected by changes in its sedimentation properties (8). The folded chromosome system has been used successfully to demonstrate the ability of *E. coli* to repair double-strand breaks (22). Because the nucleoid integrates the relationship between DNA and other macromolecular components, and because of the multitarget nature of heat, the folded chromosome system seems ideal to study the damaging effects of heat. This work explores the effects that thermal damage has on the nucleoid of *E. coli*.

MATERIALS AND METHODS

Chemicals and enzymes. Sarkosyl NL-95 (sodium *N*-lauroylsarcosinate) was a gift of CIBA-GEIGY (Dyestuffs and Chemicals Division, Greensboro, N.C.). Fluorinert FC-48 was a gift of the 3M Co. (Commercial Chemicals Division, St. Paul, Minn.). Angio-CONRAY (sodium iothalamate) was purchased from Mallinckrodt, Inc. (Pharmaceutical Division, St. Louis, Mo.). Sucrose was density gradient grade (RNase free), obtained from Schwarz/Mann (Orangeburg, N.Y.). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). [*Methyl*-^{3}H]thymidine (40 Ci/mmol), [*methyl*-^{14}C]thymidine (47.5 mCi/mmol), L-[4,5-^{3}H]leucine (60 Ci/mmol), [2-^{3}H]glycerol (200 mCi/mol), [5-^{3}H]uracil (30 Ci/mmol), Aquasol, and mini-vials were all purchased from New England Nuclear Corp. (Boston, Mass.).

Egg-white lysozyme (EC 3.2.1.17; muramidase; grade I; 3× crystallized; 38,500 U/mg) was obtained from Sigma Chemical Co. Beef pancreas DNase I was obtained from Miles Laboratories (Elkhart, Ind.).

Microorganism. The bacterial strain used in this study was a derivative of *E. coli* K-12 originating from the culture collection of Howard Flanders and desig-

† Contribution no. 3854 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

‡ Present address: Genex Corp., Rockville, MD 20852.

nated AB1157 (*arg, his, leu, pro, thr, thi*) wild type for repair abilities (9, 10); it was obtained from D. Botstein, Biology Department, Massachusetts Institute of Technology, as strain D5608. The strain employed in this study was a *thy* derivative of D5608. A trimethoprim selection (15) was used to obtain strain KU105, capable of growing at a thymine concentration of 2 μg/ml (low level thymine requirement).

Media and buffer solutions. M9 buffer contained the following salts per liter: Na_2HPO_4, 7 g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g. Luria broth agar contained 10 g of Tryptone, 10 g of NaCl, 5 g of yeast extract, and 15 g of agar per liter. All of these components were obtained from Difco Laboratories (Detroit, Mich.). Phosphate buffer (0.067 M; pH 7) contained 3.5 g of $NaH_2PO_4 \cdot H_2O$ and 5.79 g of Na_2HPO_4 per liter.

Culture conditions. A defined medium for overnight growth of cultures was prepared by combining the stock solutions immediately before use. The defined medium was made by combining 10 ml of double-strength M9 buffered salts, 10 ml of an amino acid solution containing 2×10^{-3} M L-arginine, L-histidine, L-leucine, L-proline, L-threonine, 0.15 ml of 40% glucose, 0.1 ml of a thiamine solution (0.1 mg/ml filter sterilized) and 0.2 ml of thymine solution (1 mg/ml).

Overnight cultures were prepared by inoculating 20 ml of defined medium from Luria broth agar plates and incubating for 12 to 18 h at 37°C with constant shaking (220 rpm). They were subcultured (1:100) into 20 ml of defined medium, containing the appropriate radiochemicals in a side-arm (Erlenmeyer) flask and incubated at 37°C with constant shaking (150 rpm).

Radioactive labeling of cultures. Cultures were labeled in a medium similar to the overnight defined growth medium except that it contained 0.25 ml of the 40% glucose solution and 10 μl of the thymine solution. For leucine-thymidine dual labeling, the culture medium had 1/10 of the normal amount of unlabeled leucine. The radiochemicals were added to the growth medium when subcultured, and the labeling was continued for 4 h, by which time the culture had reached early exponential phase.

The amounts of radiochemicals per 20 ml of medium were: 200 μl (20 μCi) of [^{14}C]thymidine; 50 μl (50 μCi) of [^{3}H]thymidine; 100 μl (100 μCi) of [^{3}H]glycerol; 200 μl (200 μCi) of [^{3}H]leucine; and 200 μl (200 μCi) of [^{3}H]uracil.

Heat treatment. When cultures had reached early exponential phase (4 h at 37°C), the 300-ml side-arm flask was shifted to a water bath equilibrated at 50°C (±0.2°C) (150 rpm; heating-up time was 2 to 3 min). After heat treatment for various time periods, the cultures were cooled in ice and harvested by centrifugation (7,500 × *g*, 5 min; 4°C). Preliminary experiments demonstrated that this procedure did not affect the sedimentation behavior of nucleoids compared to those obtained from cells that were not chilled. The supernatant was decanted and the pellet was suspended in solution A (composition given below) to begin the treatment to isolate the nucleoids.

Isolation of folded chromosomes. In the normal procedure, after harvesting the heated cells (~4×10^9 cells), the pellet was suspended in 3 ml of 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). Two-tenths of this cell suspension was placed in glass test tubes (12 by 75 mm) and held at 0°C. A 50-μl volume of solution B at 0°C was then added to the glass test tubes and mixed gently on a Vortex-Genie. Solution B contained 0.12 M Tris-hydrochloride buffer (pH 8), 50 mM EDTA, and 4 mg of egg-white lysozyme per ml that was freshly prepared at least once a week. Solutions A and B were stored at 4°C.

If isolation of membrane-free nucleoids was sought, the glass test tubes were transferred to a water bath at 25°C for 5 min with occasional swirling. If isolation of membrane-associated nucleoids was sought, incubation at 0°C for 6 min was performed.

In the first case, after incubation at 25°C for 5 min, the test tubes were returned to 0°C for 1 min before the addition of 0.25 ml of solution C at 0°C. Solution C contained 1% (wt/vol) Brij-58 (polyoxyethylene 20-cetyl ether), 0.4% (wt/vol) sodium deoxycholate, 2% (wt/vol) Sarkosyl NL-97 (sodium *N*-lauroylsarcosinate), 2.0 ml of NaCl, and 10 mM EDTA (pH 8.2). Solution C was prepared biweekly and stored at 4°C. In the second case, the solution employed was named C′ and was the same solution as solution C but without Sarkosyl NL-97.

After the addition of solution C or C′ at 0°C, the test tubes were swirled gently to mix the detergent mixture with the spheroplasting suspension. The turbid solution became clear after several seconds but was held at 0°C for an additional 15 min. A 0.1-ml volume of the lysate was then gently layered on the sucrose gradients with a Clay-Adams pipette fitted with a wide-bore plastic tip. The plastic tips were obtained by cutting 4-cm sections from the delivery end of disposable 1-ml Falcon pipettes (inside diameter 2.0 mm).

Treatment of nucleoids with DNase. After the cells were treated with solutions A, B, and C, 0.4 ml of the cell lysate was treated with 50 μg of DNase I per ml for 2 h at 37°C. A 0.1-ml volume of the lysate was then gently layered on the sucrose gradients.

Sucrose gradient centrifugation. The 10 to 50% (wt/vol) sucrose gradients were prepared with gradient solutions containing 1.0 M NaCl, 0.01 M Tris-hydrochloride buffer (pH 8.2), 1 mM EDTA, and density gradient grade (RNase-free) sucrose. These 10 to 50% sucrose gradients were employed to be able to observe the presence of membrane-free and membrane-associated nucleoids. The gradients were made individually at room temperature using a Buchler gradient mixing chamber and pump. A 0.3-ml high-density shelf composed of 50% (wt/vol) sucrose dissolved in 18% sodium iothalamate solution was placed on the bottom of the centrifuge tubes before forming the 4.8-ml, 10 to 50% sucrose gradients. All gradients were made in untreated cellulose nitrate centrifuge tubes (0.5 by 2 inch [1.27 by 5.08 cm]) and cooled to 4°C for at least 2 h before use.

Sometimes, before the gradients were centrifuged, 10 μl of [^{14}C]thymine-labeled T4 phage was added to the top of the gradients. The T4 phage served as a sedimentation marker with a sedimentation coefficient of 1,025S (4). Gradients were centrifuged in an SW50.1 swinging-bucket rotor in a Beckman L5-75 ultracen-

trifuge at 4°C. To minimize any rotor-speed effects (8, 17), the rotor speed employed was usually 3,000 or 4,000 rpm (except when the rotor speed effect itself was being investigated). The final $\omega^2 t$ was 6×10^9 rad^2/s.

Gradients were fractionated from the top using an ISCO Model 640 density gradient fractionator. Fluorinert FC-48, a dense, nonmiscible chase solution, was pumped into the centrifuge tube through the bottom at a flow rate of 1 ml/min. Fractions (0.2 ml) were collected into Beckman mini-vials and counted in a Beckman LS-230 ambient temperature scintillation counter after adding 3 ml of Aquasol (plus 10% [wt/vol] water) per fraction. ^{3}H was counted at an efficiency of 40% and ^{14}C was counted at an efficiency of 90%.

At times, minor deviations from the procedures stated above were introduced in the experimental protocol. Where appropriate, these procedural changes are explained in Results.

RESULTS

Survival curve. The survival curve for heat-treated (50°C) *E. coli* K-12 KU 105 is shown in Fig. 1. The rate of loss of colony-forming ability on Luria broth agar was exponential.

In vivo heat-treated nucleoids. Figure 2 shows the patterns obtained when exponentially growing cells were heated at 50°C and lysed, and their nucleoid sedimentation profiles were analyzed. It was observed that heating for 15 and 30 min caused an increase in the sedimentation coefficient of the nucleoids. The nucleoids from the unheated cells had a sedimentation coefficient of approximately 1,800S at a rotor speed of 4,000 rpm. The profiles of nucleoids from heated cells showed sedimentation coefficients ranging from those observed in normal nucleoids (1,800S) to about 4,200S with a peak at 3,700S. The fast-sedimenting nucleoids were also ac-

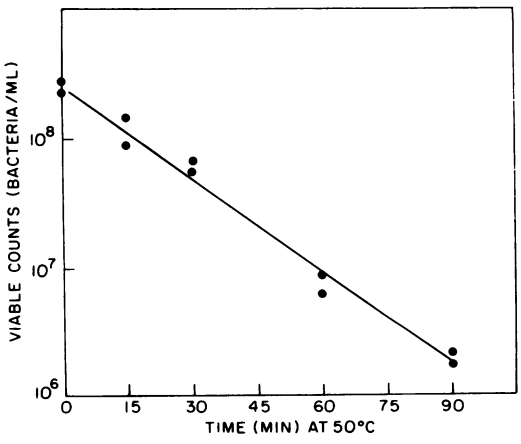

FIG. 1. *Survival curve for heat-treated Escherichia coli K-12. Exponential-phase cultures were heated at 50°C for different time periods as described in the text. For each time, portions were removed, diluted in phosphate buffer, and plated in duplicate on Luria broth agar plates (0.1 ml per plate). The plates were incubated at 37°C for 48 h before counting colonies. Duplicate points at each time interval represent the results of two separate experiments.*

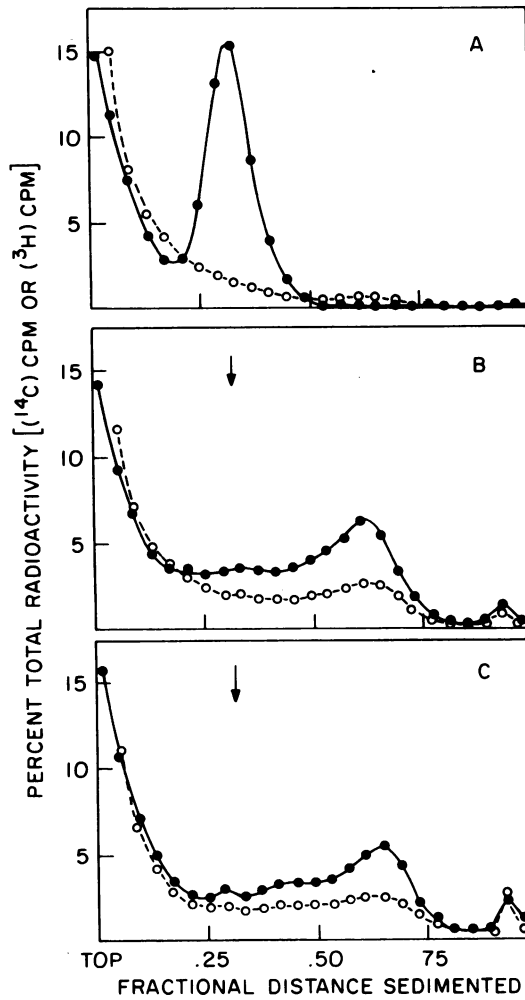

FIG. 2. *Gradient profiles for nucleoids from heated cells. The culture was labeled with [^{14}C]thymidine and [^{3}H]leucine during the exponential phase and then shifted to 50°C for different time periods, harvested by centrifugation at 4°C, and lysed. Centrifugation of the lysate in 10 to 50% neutral sucrose gradients was performed at 4°C and 4,000 rpm for a total $\omega^2 t$ of 6×10^9 rad^2/s. The total radioactivity recovered from the gradients was: (A) unheated cells (^{14}C = 6,400 cpm; ^{3}H = 135,000 cpm); (B) 50°C, 15 min (^{14}C = 9,400 cpm; ^{3}H = 211,000 cpm); (C) 50°C, 30 min (^{14}C = 7,800 cpm; ^{3}H = 173,000 cpm). The arrows in B and C indicate the position of the normal nucleoid peak. Symbols: ●, [^{14}C]thymidine; ○, [^{3}H]leucine.*

companied by protein with high sedimentation coefficients.

In Fig. 2B and C, the nucleoid profiles showed maxima at fractional distances sedimented of 0.62 and 0.66, respectively. At these maxima the ratios of ^3H:^{14}C cpm were 9.5 and 10.2, respectively. In contrast, in Fig. 2A, the ^3H:^{14}C ratio at the maximum of the nucleoid profile (fractional distance sedimented = 0.33) was 2.3. This indicated that the nucleoids from heated cells cosedimented with more protein than did nucleoids from control (unheated) cells. The profiles of DNA and protein obtained for nucleoids from cells heated for 60 min at 50°C were identical to those from cells heated for 30 min at the same temperature.

The amount of ^3H radioactivity in the first fraction is not shown in Fig. 2 because the amount of radioactivity in this fraction is very large and would fall off the scale shown in the ordinate. This amount of ^3H is due to unincorporated [^3H]leucine. In experiments in which unincorporated material was chased to polymeric form, the radioactivity in the first few fractions became negligible. These comments also apply to Fig. 3, 4, and 5. The same experiment was performed at a rotor speed of 3,000 rpm. The resulting profiles, obtained from cells heated for 30 min at 50°C, are shown in Fig. 3. At this lower rotor speed, the nucleoids (^3H radioactivity) appeared heavier than those observed at a rotor speed of 4,000 rpm. At 3,000 rpm, the peak of the profile was 4,200S. In addition, the population of nucleoids from heated cells obtained at 3,000 rpm appeared more homogeneous than those obtained at 4,000 rpm. That is, the broadness of the peak decreased at the lower sedimentation speed. It was also evident that the protein (^3H radioactivity) distribution at 3,000 rpm was different from that observed at 4,000 rpm. At 3,000 rpm, the protein profile was sharper than at 4,000 rpm.

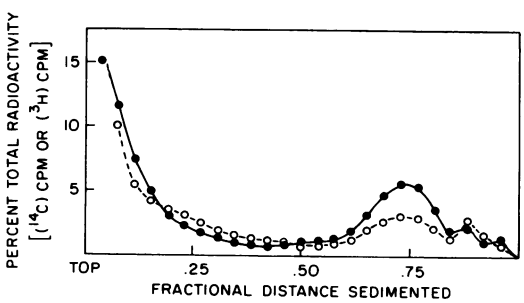

FIG. 3. *Gradient profiles of nucleoids from cells heated at 50°C for 30 min. Protocol and symbols are the same as in Fig. 2 except that centrifugation of the gradient was done at 3,000 rpm for a total $\omega^2 t$ of 6 × 10^9 rad^2/s.*

The ^3H:^{14}C cpm ratio at the maximum of the profile (fractional distance sedimented = 0.74) was 8.0. It should also be mentioned that the profiles of nucleoids from control cells were found not to be a function of the rotor speed in the range of 3,000 to 4,000 rpm. This finding demonstrated that the speed dependence of the nucleoids from heated cells was greater than that of the nucleoids from control cells.

Association of nucleoids from heated cells with different macromolecules. The results presented in Fig. 2 and 3 indicated that nucleoids from heated cells cosedimented with proteins. Furthermore, it was evident from Fig. 2 that the relative amount of protein cosedimenting with nucleoids increased after heat treatment. It thus became necessary to determine if the protein simply cosedimented with the nucleoids or if it was actually associated with the nucleoids. Accordingly, cells were heated at 50°C for 30 min, as before, and after lysis of the protoplasts the lysate was treated with DNase before sedimentation analysis. The profile obtained from this preparation (results not shown) not only showed no large-molecular-weight DNA, but also no high-sedimentation-coefficient proteins. All radioactive materials remained at the top of the gradient. This indicated that indeed the protein was associated with the nucleoids.

We also checked to see if membrane associated with the nucleoids from heated cells was present. [^3H]Glycerol was used to label phospholipids in the membrane, and a lysis procedure, which resulted in both membrane-free and membrane-associated nucleoids from control cells, was employed (12). We determined that the protein-associated nucleoids from heated cells isolated using Sarkosyl during lysis did not contain any significant amount of membrane phospholipids (Fig. 4A) compared to the amount of phospholipids found in the membrane-associated nucleoids of control cells lysed without Sarkosyl (Fig. 4B). The membrane-associated nucleoids from control cells (^{14}C; Fig. 2B) showed a maximum at a fractional distance sedimented of 0.62 and the membrane-free nucleoids sedimented at 0.29. The profile of nucleoids from heated cells (^{14}C; Fig. 2A) had a maximum at 0.64. The ^3H:^{14}C cpm ratios at the maxima in Fig. 2A (fractional distance sedimented = 0.64) and in Fig. 2B (fractional distance sedimented = 0.62) were 0.97 and 9.9, respectively. Furthermore, when the heated cells were lysed without Sarkosyl (Fig. 4C), the profile obtained showed a peak at 6,000S. The ^3H:^{14}C ratio at this maximum was 4.6. These nucleoids

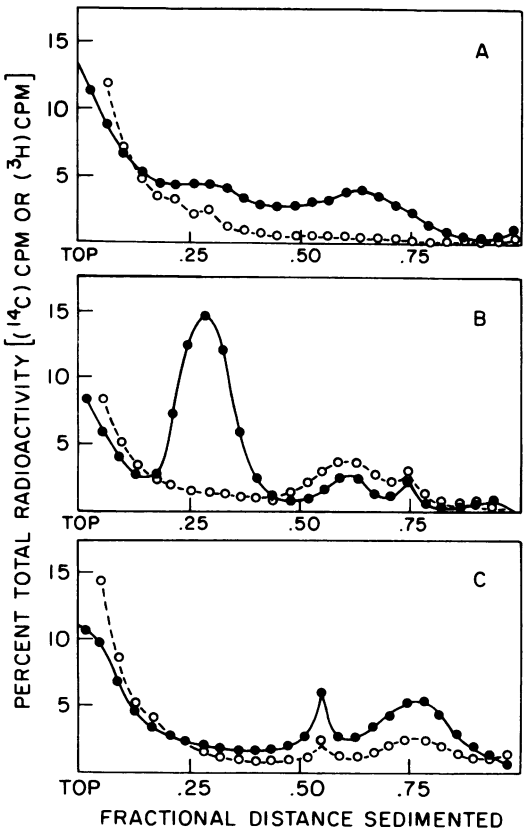

FIG. 4. *Gradient profiles of membrane-free nucleoids from heated cells and of membrane-associated nucleoids from control and heated cells. (A) Heated cells (50°C, 30 min). Lysis procedure was done with Sarkosyl. Total ^{14}C in gradient = 20,000 cpm; total ^{3}H in gradient = 70,000 cpm. (B) Control cells. Lysis procedure resulted in a mixture of membrane-free and membrane-associated nucleoids. Total ^{14}C in gradient = 22,000 cpm; total ^{3}H in gradient = 93,000 cpm. (C) Heated cells (50°C, 30 min). Lysis procedure as in B. Total ^{14}C in gradient = 17,000 cpm; total ^{3}H in gradient = 63,000 cpm. Centrifugation of the lysates was at 4,000 rpm for a total $\omega^2 t$ of 6 × 10^9 rad^2/s. Symbols: ●, [^{14}C]thymidine; ○, [^{3}H]glycerol.*

were heavier than both the nucleoids from heated cells isolated with Sarkosyl (membrane-free nucleoids) and the nucleoids isolated from control cells without Sarkosyl (membrane-associated nucleoids). This finding indicated that the increase in sedimentation coefficient of the nucleoids due to heat treatment was above and beyond that which could be due to the presence of membranes. Thus, we concluded that the increase in the sedimentation coefficient due to heat treatment is not the consequence of the presence of membrane in the nucleoids from heated cells.

The presence of RNA in heated nucleoids was also investigated. Cultures labeled with [^{3}H]uracil and [^{14}C]thymidine were subjected to heat treatment, and the nucleoid profiles were analyzed. We were not able to detect any significant increases in the amount of ^{3}H radioactivity associated with nucleoids isolated from the heated cells (results not shown).

Reconstruction experiments. To determine if the interaction between the nucleoids and cellular proteins from heated cells occurred as a result of the lysis procedure, or if it was a prelysis event, reconstruction experiments were conducted. Figure 5A shows the nucleoid gradient profiles obtained when control cells (not heated) were mixed with heated cells and then lysed together. No interaction between nucleoids from heated cells and nucleoids from control cells was seen. Figure 5B and C show that there was no interaction between the proteins from control cells and nucleoids from heated cells, and vice versa, when the cells were mixed before lysis.

DISCUSSION

The data presented in the previous section show that nucleoids from heated cells exhibited a higher sedimentation coefficient than nucleoids from control cells. In addition, the nucleoids from heated cells appeared to be closely associated with cellular protein. That is, heat treatment caused an increase in the amount of protein found associated with the nucleoids.

The fact that the treatment of nucleoids from heated cells with DNase resulted in loss of both high-sedimentation-coefficient DNA and protein indicated that the protein did not simply cosediment with DNA but rather that it was closely associated with it. The experiments with cells labeled with [^{3}H]uracil and [^{3}H]glycerol showed that the nucleoids from heated cells did not contain larger amounts of RNA associated with them than nucleoids from control cells and also that they did not contain membrane fractions.

The reconstruction experiments demonstrated that neither the protein nor the DNA from control cells interacted with the same components from heated cells during lysis. This finding indicated that the association of cellular protein occurs in vivo during heating.

The increase in sedimentation coefficient of nucleoids from cells after heat treatment was probably due, in part, to the increase in mass which the protein causes.

The sedimentation coefficients of nucleoids from heated cells were also shown to be more speed dependent than nucleoids from control

cells. This conclusion, drawn from the data shown in this paper (for speeds of 3,000 to 4,000 rpm), is also supported by experiments not shown here, in which the nucleoid profiles of control and heated cells were also analyzed at 6,000, 7,000, 17,000, and 30,000 rpm. As the rotor speed was increased, the nucleoids from heated cells showed progressively lower sedimentation coefficients than control cells. At 7,000 rpm, nucleoids from cells heated at 50°C for 30 min had a very sharp profile with a peak at approximately 1,100S. Above 7,000 rpm, the nucleoids from heated cells aggregated and were found in one or two fractions, the apparent sedimentation coefficient being 1,000S or less. This strong dependence of sedimentation coefficient on rotor speed of nucleoids from heated cells indicated unfolding of the structure.

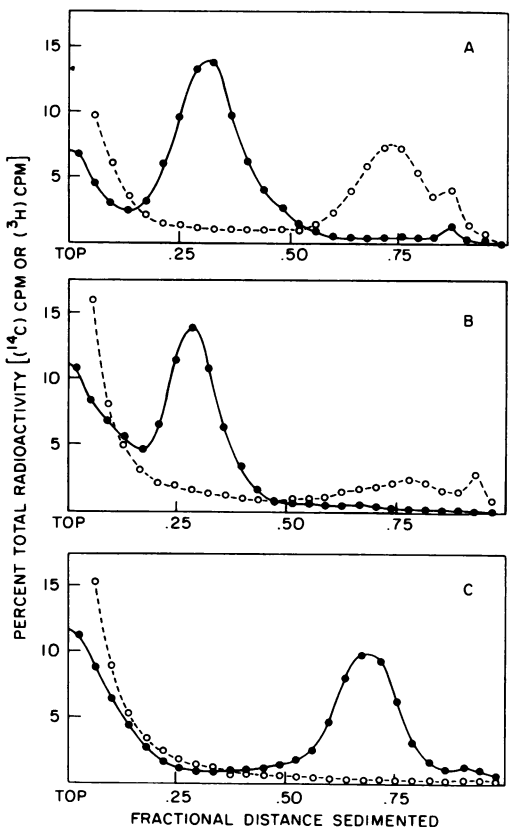

FIG. 5. *Reconstruction experiments. (A) Control cells labeled with [^{3}H]thymidine (—●—) were mixed with heated cells (30 min at 50°C) labeled with [^{14}C]thymidine (- - -○- - -). The mixture was lysed and subjected to sedimentation analysis. Normal lysis procedure, yielding membrane-free nucleoids exclusively, was used. Centrifugation was at 3,000 rpm for a total of 6 × 10^{9} rad^{2}/s. (B) Control cells labeled with [^{14}C]thymidine (—●—) were mixed with heated cells (30 min at 50°C) labeled with [^{3}H]leucine (- - -○- - -). The mixture was lysed and subjected to sedimentation analysis as in A. (C) Heated cells (30 min at 50°C) labeled with [^{14}C]thymidine (—●—) were mixed with control cells labeled with [^{3}H]leucine (- - -○- - -). The mixture was lysed and subjected to sedimentation analysis as in A.*

Heat-induced DNA breakages in *E. coli* are well documented in the literature (5, 19, 20, 23). It is also known that whereas DNA breakage decreases the sedimentation rate in the nucleoids (18), it does not increase speed dependence (8). It is very likely that nucleoids from cells heated for 15, 30, and 60 min contained DNA breaks, and any effects that these may have had on sedimentation were masked by the unfolding of the nucleoid structure and the protein associated with it.

The sedimentation coefficients of nucleoids from heated cells are dependent on at least two effects. The first is the association of cellular protein with the nucleoids, which will increase their mass and therefore their sedimentation coefficients. The second is the destabilization of the nucleoid structure, resulting in an increased speed dependence. The latter will result in lower sedimentation coefficients as the centrifugation speed is increased. As for the cause of the speed dependence, we cannot rule out the possibility that the protein associated with the nucleoids is the factor responsible for the speed dependence and that unfolding of the structure is not involved. The overlapping of these two opposite driving forces for sedimentation rate (protein association and structural destabilization) would result in nucleoids containing similar amounts of protein but different degrees of unfolding sedimenting at different sedimentation coefficients (Fig. 2B and C). The ratios of leucine to thymidine in fractions spanning sedimentation coefficients from 2,360S to 3,970S in Fig. 2B and C are quite similar (Table 1).

In conclusion, we have shown that the structure of the nucleoids from heated *E. coli* is modified in such a way that their sedimentation coefficient becomes extremely speed dependent and also is greater than those observed in nucleoids from control cells. The increase in sedimentation coefficient is most likely due, in part, to the cellular protein that associates with the nucleoids as a result of the heat treatment. The speed dependence could be due either to the protein itself or to the unfolding of the structure. At any rate, these changes are probably incompatible with viability and would have to be reversed if a cell is to survive. The ability of cells to repair these damages and recover their viability will be the subject of future studies.

TABLE 1. *Ratios of leucine to thymidine in the sedimentation profiles of cells heated at 50°C for 15 and 30 min*[a]

Fractional distance sedimented	S[b]	Ratio of [^{3}H]leucine (cpm) to [^{14}C]thymidine (cpm)	
		15 min	30 min
0.41	2,360	11.7	13.3
0.45	2,590	10.9	13.4
0.49	2,800	10.7	13.8
0.53	3,040	10.4	13.0
0.57	3,260	10.2	11.9
0.61	3,490	9.5	11.2
0.65	3,740	10.1	10.2
0.69	3,970	11.9	11.4

[a] See Fig. 2B and C.

[b] Sedimentation coefficient calculated as described in Ulmer et al. (22).

ACKNOWLEDGMENTS

This work was supported by grant DAAG-29-76-C-0034 from the United States Army Research Office. Jose R. Pellon was supported by a fellowship from the Instituto Tecnologico de Postgraduados, Madrid, Spain.

LITERATURE CITED

1. **Beuchat, L. R.** 1978. Injury and repair of gram negative bacteria, with special consideration of the involvement of the cytoplasmic membrane. Adv. Appl. Microbiol. **23**:219-243.
2. **Bridges, B. A., M. J. Ashwood-Smith, and R. J. Munson.** 1969. Susceptibility of mild thermal and of ionizing radiation damage to the same recovery mechanisms in *Escherichia coli*. Biochem. Biophys. Res. Commun. **35**: 193-196.
3. **Bridges, B. A., M. J. Ashwood-Smith, and R. J. Munson.** 1969. Correlation of bacterial sensitivities to ionizing radiation and mild heating. J. Gen. Microbiol. **58**: 115-124.
4. **Cummings, D.** 1964. Sedimentation and biological properties of T-even phage of *Escherichia coli*. Virology **23**: 408-418.
5. **Gomez, R. F.** 1977. Nucleic acid damage in thermal inactivation of vegetative microorganisms. Adv. Biochem. Eng. **5**:49-67.
6. **Grecz, N., and S. Bhatarakamol.** 1977. Apurinic acid endonuclease implicated in DNA breakage in *Escherichia coli* subjected to mild heat. Biochem. Biophys. Res. Commun. **77**:1183-1188.
7. **Hecht, R. M., and D. E. Pettijohn.** 1976. Studies of DNA bound RNA molecules isolated from nucleoids of *Escherichia coli*. Nucleic Acids Res. **3**:767-788.
8. **Hecht, R. M., D. Stimpson, and D. E. Pettijohn.** 1977. Sedimentation properties of the bacterial chromosome as an isolated nucleoid and as an unfolded DNA fiber: chromosomal DNA unfolding measured by rotor speed effects. J. Mol. Biol. **111**:257-277.
9. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119-1136.
10. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137-1150.
11. **Hurst, A.** 1977. Bacterial injury: a review. Can. J. Microbiol. **23**:935-944.
12. **Korch, C., S. Ovrebo, and K. Kleppe.** 1966. Envelope associated folded chromosome from *Escherichia coli*: variation under different physiological conditions. J. Bacteriol. **127**:935-944.
13. **Materman, E. C., and A. P. Van Gool.** 1978. Nucleoid release from *Escherichia coli* cells. J. Bacteriol. **133**: 878-883.
14. **Materman, E. C., and A. P. Van Gool.** 1978b. Compact *Escherichia coli* nucleoids in a highly supercoiled conformation. J. Bacteriol. **135**:703-706.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Pettijohn, D. E.** 1976. Prokaryotic DNA in nucleoid structure. CRC Crit. Rev. Biochem. **4**:175-202.
17. **Pettijohn, D., R. M. Hecht, D. Stimpson, and S. van Scoyk.** 1978. An explanation for rotor speed effects observed during sedimentation of large folded DNA molecules. J. Mol. Biol. **119**:353-359.
18. **Pettijohn, D. E., R. M. Hecht, O. G. Stonington, and T. D. Stamato.** Factors stabilizing DNA folding in bacterial chromosomes, p. 145-162. *In* R. D. Wells and R. B. Inman (ed.), DNA synthesis *in vitro*. University Park Press, Baltimore.
19. **Pierson, M. D., R. F. Gomez, and S. E. Martin.** 1978. The involvement of nucleic acids in bacterial injury. Adv. Appl. Microbiol. **23**:263-285.
20. **Sedgwick, S. G., and B. A. Bridges.** 1972. Evidence for indirect production of DNA strand scissions during mild heating of *Escherichia coli*. J. Gen. Microbiol. **71**:191-193.
21. **Ulmer, K. M., R. F. Gomez, and A. J. Sinskey.** 1979. Ionizing radiation damage in the folded chromosome of *Escherichia coli* K-12: sedimentation properties of irradiated nucleoids and chromosomal deoxyribonucleic acid. J. Bacteriol. **138**:475-485.
22. **Ulmer, K. M., R. F. Gomez, and A. J. Sinskey.** 1978. Ionizing radiation damage to the folded chromosome of *Escherichia coli* K-12: repair of double-stranded breaks in deoxyribonucleic acid. J. Bacteriol. **138**:486-491.
23. **Woodcock, E., and G. W. Grigg.** 1972. Repair of thermally-induced DNA breakage in *Escherichia coli*. Nature (London) New Biol. **237**:76-79.