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Centrifugal Field Effects on the Sedimentation Coefficient of the *Escherichia coli* Nucleoid After Heat Treatment

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The effects of the centrifugal field on the sedimentation coefficient of the heated (50° C, 30 min) *Escherichia coli* nucleoid were investigated. From 3,000 r.p.m. the sedimentation coefficients of the heated nucleoids were highly dependent on rotor speed. At 3,000 r.p.m. their sedimentation coefficient was about 4,000 S while at 7,000 r.p.m. it was about 1,500-1,700 S. At 7,000 r.p.m. and over, nucleoid aggregations occurred and it was difficult to differentiate speed dependence from nucleoid aggregation. Factors likely to cause speed dependence and/or nucleoid aggregation are indicated. The practical importance of these findings is pointed out.

The dependence of the sedimentation coefficient (sedimentation rate per unit centrifugal field) on the centrifugal field has been observed in high molecular weight unfolded DNA by a number of authors (8, 9, 12, 21, 22). This phenomenon has been termed speed dependence and it results in low sedimentation coefficients at high rotor speeds while at low rotor speeds the sedimentation coefficient gradually reaches a maximum value. ZIMM (27) proposed a theory based on the different frictional effects at the ends of a large DNA polymer compared with the more shielded middle segments. During centrifugation the ends would tend to drag behind resulting in decreased sedimentation coefficients at high rotor speeds. The magnitude of the distortion would be dependent on both the centrifugal force and the end-to-end distance or length of the DNA polymer. CHIA and SCHUMAKER (3) pointed out the importance of using low centrifugal fields, showing that a low molecular weight DNA can sediment faster than a higher molecular weight DNA if suficiently high rotor speeds are employed.

Considering that the nucleoid or folded chromosome is a compact particle-like structure (low specific viscosity and high sedimentation rate), the speed dependence effect was not expected to apply to nu-

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cleoids. Contrary to expectations, HECHT *et al.* (7) showed that the sedimentation coefficient of the isolated nucleoid of *Escherichia coli* depended on the strength of the centrifugal field. The membrane-free and the membrane-associated nucleoids increased their sedimentation coefficient when the centrifugal field was lowered. These changes were reversible and not due to any loss of mass in the nucleoid components. Extrapolating the rotor speed to zero, the membrane-free nucleoid had a sedimentation coefficient of 1,900S (at 30,000 rpm this sedimentation coefficient was 1,000S). The membrane-associated nucleoid has a more pronounced rotor speed effect (from 5,500S at zero rotor speed to 2,500S at 25,000 rpm). PETTIJOHN *et al.* (19) proposed a model to explain the rotor speed effect suggesting that the terminal ends of the DNA loops in the chromosome became hydrodynamically analogous to the ends of the linear DNA double-helix and were more extended at high centrifugal fields, increasing their frictional coefficient. The Zimm equation (27) when applied to the previous model showed consistency between the predicted and the observed values of the sedimentation coefficient.

Mild heat treatments (50° C) of *E. coli* cells produce the association of proteins to their nucleoids together with an increase in their sedimentation coefficient (15). This association is reversible *in vivo* after a repair period at 37° C, and the percentage of nucleoids dissociating from the protein is related to the percentage of cells able to repair the thermal damage (14). Reversible protein association to DNA after mild heat treatments (hyperthermia) has been also shown in mammalian cells (20, 24). The similar response of procaryotes and eucaryotes to heat suggests a general mechanism of response to thermal treatments. These thermal treatments (thermotherapy) have proved useful (producing cures) recently in the treatment of tumors either alone (13, 23) or in combination therapies (1, 2, 4, 10, 11).

The purpose of this article is to determine how the centrifugal field affects the sedimentation properties of the heated protein-associated nucleoid. This is of practical importance in order to draw meaningful conclusions from sedimentation analyses at specific rotor speeds. The rotor speed effect on the nucleoid has been already used to study the restraints on condensed DNA (7). This article is part of a series on the heat effects in the *E. coli* nucleoid (14-16) whose ultimate goal is to help establish the nucleoid as a useful tool in studies concerning the molecular mechanisms of DNA damage (17), particularly heat damage.

Materials and Methods

All materials and procedures have been previously described (15). The only changes are those corresponding to the centrifugation of the sucrose gradients. The gradients with the cell lysates layered on top (containing the nucleoids) were centrifuged in a SW 50.1 swinging bucket rotor in a Beckman L5-75 ultracentrifuge at 4° C. The centrifuge has a built in ω^2t integrator, which when used in the *Preset* model allowed for a constant final ω^2t (total centrifugal field) regardless of the rotor speed. The final ω^2t was always 6×10^9 rad^2/sec.

Results and Discussion

The protein-associated heated nucleoids have a higher sedimentation coefficient than the control unheated nucleoids when the rotor speed is low enough (15). For example, at 3,000 rpm the sedimentation coefficient for the control nucleoids was about 1,900 S and for the heated nucleoids about 4,000 S. At this rotor speed the sedimentation coefficient of both nucleoid populations showed a normal distribution (15). Increasing the rotor speed to 4,000 rpm caused the distribution of sedimen-

tation coefficients in heated nucleoids to range from a few hundreds to 4,000 S, while for the control nucleoids the sedimentation coefficient did not change (15).

Figure 1 shows the effect of the rotor speed (centrifugal field) over a range of 4,000 to 30,000 rpm on the sedimentation coefficient of heated (50° C, 30 min) protein-associated nucleoids. It can be observed that heated nucleoids are extremely speed dependent. At 4.000 rpm their sedimentation coefficient ranged from a few hundreds to about 4,000 S, while at speeds of 7,000 rpm or more it decreased dramatically and the peak in the sedimentation profile became very sharp. These sharp peaks, that usually are recovered in only one fraction (one point in the profile), consist of nucleoid aggregations and sometimes can be seen as white bands in the centrifugue tubes. These fractions have high viscosity (visually determined) indicative of unfolding and loss of tertiary structure of the DNA in the nucleoid.

The results in figure 1 and the previously reported speed dependence of heated nucleoids in the range of 3,000-4,000 rpm (15) demonstrate that in order to study structural changes in heated nucleoids, by rate zonal sedimentation in neutral sucrose gradients, rotor speeds close to 3,000 rpm should be employed. If speed dependence is to be used as a tool to study the restraints on DNA, rotor speeds from 2,500 to 4,000 rpm need to be employed. This is due to the fact that at over 4,000 rpm it is difficult to differentiate speed dependence from nucleoid aggregation. These numbers would change with the amount of nucleoids loaded in the gradient and with the heat treatment applied as shown below.

The effect of heating time on the sedimentation coefficient of the nucleoids at a rotor speed of 7,000 rpm is shown in figure 2. As we have seen before, at 7,000 rpm it is difficult to differentiate speed dependence from nucleoid aggre-

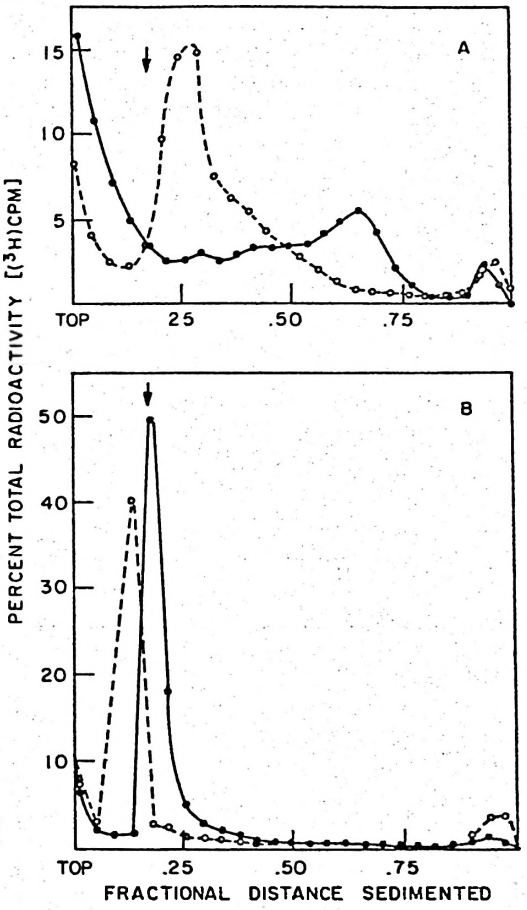

Fig. 1. *Gradient profiles for nucleoids from heated cells at various centrifugation speeds.* The cultures were labeled with ^{3}H-thymidine and in early exponential phase they were shifted to a 50° C bath for 30 minutes, harvested, the cells lysed to obtain their nucleoids and loaded on 10-50 % sucrose gradients (15). The centrifugation was carried out at 4° C for a total $\omega^2 t$ of 6×10^9 rad^2/sec at different rotor speeds.

A: ●—● Centrifugation at 4,000 rpm (^{3}H = 76,000 CPM).
O----O Centrifugation at 7,000 rpm (^{3}H = 71,000 CPM).
B: ●—● Centrifugation at 17,000 rpm (^{3}H = 79,000 CPM).
O----O Centrifugation at 30,000 rpm (^{3}H = 54,000 CPM).

The arrows in the upper left indicate the position of a T-4 marker phage (1,025S) (25).

gation. This figure serves to make a very important practical point. Assume we are trying to deduce structural changes occurring in nucleoids from heated cells by sedimentation analyses; under these conditions (7,000 rpm) we will conclude that heating produced a decrease in the nucleoid sedimentation coefficient probably due to some unfolding or breakage of the DNA. As we have previously shown (15) this is not the case. On the contrary, heat treatment increases the sedimentation coefficient of the nucleoids. In figure 2, a differential effect dependent upon treatment time from 15 to 60 min would be inferred and this is not so at lower rotor speeds like 4,000 or 3,000 rpm (15), that is, at low rotor speed increasing heating time from 15 to 60 min does not affect the sedimentation coefficient. From figure 2 we can conclude that the heating time increases the tendency of the nucleoids to aggregate. The relative contribution of aggregation and speed effects at this speed (7,000 rpm) is, however, not clear at this point.

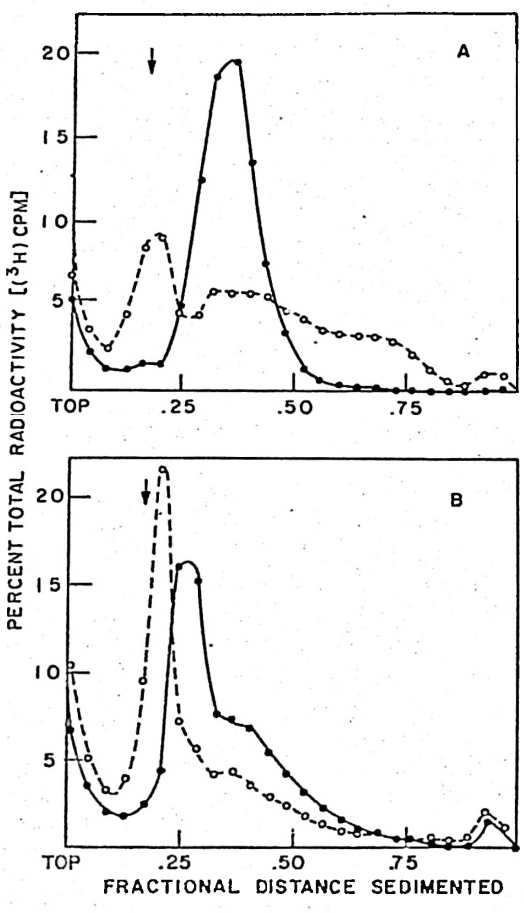

Fig. 2. *Gradient profiles for nucleoids from heated cells (50° C; 15, 30, 60 min) centrifuged at 7,000 rpm.*

The culture was labeled with ^{3}H-thymidine as usual and the experimental procedure was the same as that in figure 1, except that the centrifugation of the lysate on the 10-50 % sucrose gradients was carried out at 7,000 rpm.

A: ●—● Untreated cells (^{3}H = 75,000 CPM).
O----O 50° C, 15 min (^{3}H = 78,000 CPM).
B: ●—● 50° C, 30 min (^{3}H = 79,000 CPM).
O----O 50° C, 60 min (^{3}H = 72,000 CPM).

The arrows in the upper left indicate the position of a T4 marker phage (1,025S).

The DNA folds or domains in the nucleoid are thought to be stabilized by RNA-DNA interactions (7, 15, 18) and only proteins that bind RNA to DNA (like RNA polymerase) could represent an additional joining force. The unfolding of the DNA decreases the nucleoid sedimentation coefficient and increases its speed dependence. *In vitro*, the temperature at which half of the nucleoids are partially unfolded is 55° C (5) in a solvent containing 0.1 M NaCl and 2×10^{-3} M spermidine (which is the intracellular concentration). This unfolding is probably due to the release of the RNA bound to DNA (6). As we have already reported (15) this could also be the case *in vivo*, where DNA unfolding and protein association are the probable cause for the dramatic speed dependence of the sedimentation coefficient of the heated nucleoids. *In vivo*, mild heat (~50° C) also produces DNA breakage (26) and consequently relaxation of DNA supercoiling (25), which in turn results in a reduction in the nucleoid sedimentation coefficient but not in an increased relative speed de-

pendence of the sedimentation coefficient of these nucleoids. Since DNA breakage produces an increase of free ends, this fact together with the unfolding and protein association is likely to be an important factor in the production of nucleoid aggregates.

Resumen

Se estudia la influencia del campo centrífugo en el coeficiente de sedimentación de los nucleoides o cromosomas plegados de *Escherichia coli* después de un tratamiento térmico a 50° C durante 30 minutos. De 3.000 a 7.000 rpm los coeficientes de sedimentación de los nucleoides calentados resultan ser altamente dependientes de la velocidad de centrifugación. A 3.000 rpm su coeficiente de sedimentación es de 4.000S, mientras que a 7.000 rpm es aproximadamente de 1.500-1.700S. La centrifugación a 7.000 o más rpm origina agregaciones de nucleoides, haciendo difícil la separación entre la dependencia de la velocidad de centrifugación y la agregación. Se indican las posibles causas de la dependencia de la velocidad de centrifugación y/o de la agregación. La importancia práctica de estos resultados reside en el hecho de que para extraer conclusiones de los análisis de sedimentación a una velocidad de centrifugación determinada se debe saber previamente cuál es el efecto de la velocidad de centrifugación en las propiedades de sedimentación del nucleoide.

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