SHEAR AND THE MELTING OF DNA: AN ESPECIALLY SENSITIVE PORTION OF THE *E. COLI* GENOME

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ABSTRACT The melting point of DNA is shown to be a function of shear stress. The higher the molecular weight of the DNA, the further its melting point is lowered by a given shear rate. During lysis of *E. coli*, a part of the DNA is especially shear sensitive, so that its melting curve in the presence of shear shows a low-melting region prior to the main transition. Lysis and dilution of the cell contents destroys the extra shear sensitivity, perhaps because the DNA dissociates from the cell membrane or from some other large subcellular structure. Such a structure would impart increased shear sensitivity to the associated region of the genome.

INTRODUCTION

Several years ago we observed that a part of the DNA of *Escherichia coli* can be more easily denatured than the rest, during lysis (Rosenberg and Cavalieri, 1964). In order to elicit any denaturation, the cells had to be stirred during lysis at a somewhat elevated temperature. This communication demonstrates not only that the melting point of DNA is a function of shear, but also that at the moment of lysis a rather large portion of the DNA of *E. coli* is considerably more shear sensitive in its melting than is the major component. This portion of the DNA may have a particular biological role. Our earlier experiments suggested a relationship to DNA and RNA synthesis (Rosenberg and Cavalieri, 1964; Rosenberg and Cavalieri, 1968*a*). Several other investigators have also correlated a denatured fraction of DNA with DNA and/or RNA synthesis (Rolfe, 1963; Kidson, 1966; Okazaki et al., 1968). The way in which such DNA might function is not yet clear. However, the knowledge that this fraction of DNA differs from the remainder in possessing unusual shear sensitivity may aid in the determination of its biological significance.

METHODS

DNA Melting Curves

E. coli K12 DNA was purchased from General Biochemicals, Chagrin Falls, Ohio. Its sedimentation constant was 20. All solutions studied contained the following, in addition to DNA:

0.02 M tris(hydroxymethyl)-aminomethane (Tris), 0.02 M ethylenediaminetetraacetate (EDTA), 1% sodium dodecyl sulfate (SDS), pH 8.5–9.2 (pH measured at 25°C; at 100°C, the pH is still greater than 7).

The ambient melting curve was determined by heating slowly in an apparatus described elsewhere (Cavalieri, Small, and Sarkar, 1962) in a Beckman DU spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.). The optical density (OD) was read at 270 mμ; the results should not differ significantly from those at 260 mμ (Felsenfeld and Sandeen, 1962).

The nonambient OD melting curve was determined by heating aliquots for 10–20 min at various temperatures, then cooling rapidly and reading the OD at 260 mμ. Some samples were sheared, while heating, either by stirring with a magnetic bar (15 × 4 mm) in a 15 mm glass test tube at about 300 rpm, or by homogenizing in a glass apparatus consisting of closely-spaced concentric cylinders with the inner cylinder rotating at various speeds. The diameter of the inner cylinder was 1.5 cm, the height, 10 cm, and the annular space, 0.01 cm. The same cooled samples were used for CsCl density gradient centrifugation.

CsCl Density Gradient Centrifugation

The procedure used has been described elsewhere (Rosenberg and Cavalieri, 1964). The DNA concentration in all samples was about 0.01 mg/ml. The samples were banded in a Spinco Model E ultracentrifuge at 25°C (Spinco Div., Beckman Instruments, Inc.). The percentage of the total DNA banding at the density of denatured *E. coli* DNA was measured in each case.

Cell Lysis

Aliquots of exponentially-growing *E. coli* B cultures were centrifuged to give pellets containing about 3×10^9 cells. The pellets were then frozen, except where noted otherwise. When frozen, the cells were stored at −20°C and kept for no more than several days. The cells were generally lysed by first equilibrating the frozen pellet at the desired temperature, then adding 3 ml of Tris-EDTA-SDS solvent at the same temperature. Lysis occurred rapidly. The lysates were cooled after 20 min. Stirring during or after lysis was accomplished with a 15 × 4 mm magnetic bar in a 15 mm cellulose nitrate test tube at about 300 rpm (Rosenberg and Cavalieri, 1964). Lysates were diluted appropriately with 0.02 M Tris before CsCl density gradient centrifugation.

When the cells were to be heated before lysis, fresh pellets were suspended in 0.5 ml of 0.02 M Tris, pH 8.0, and heated for 20 min. at the desired temperature. After cooling, 2.5 ml of the Tris-EDTA-SDS solvent plus 0.6 mg pronase were added; incubation for 15 min. at 33° produced lysis.

When the cells were to be lysed in a concentric cylinder shearing apparatus, fresh pellets were suspended in 2.0 ml of 0.02 M Tris-0.02 M EDTA, pH 9.2, and equilibrated in the apparatus at the chosen temperature. With the outer cylinder rotating at the chosen speed, 1.0 ml of preheated 0.02 M Tris-0.02 M EDTA-2% SDS was injected into the annular space with a syringe. The lysates were removed from the apparatus and cooled after 20 min. The apparatus consisted of two Lucite cylinders, 5 cm high, with diameters 1.700 cm and 1.900 cm and an annular space of 1.000 mm. Because of inefficient mixing of the two solutions, the percentages of denatured DNA obtained by lysis in this apparatus must be considered as minimum values. Sometimes lumps of gel could still be seen in the lysates, indicating that part of the DNA had not been subjected to any shear.

There was little change in the percentage of denatured DNA obtained by lysing cells at 60°C in the above apparatus over a shear range of 480–900 sec^{-1}. This indicates that the shift in melting point is proportional to a low power of the shear stress. (The data in Table I, ob-

tained by shearing purified DNA, corroborate this.) We conclude that the effective shear developed by stirring with a magnetic bar as described earlier must be very much less than 630 sec^{-1}, since it shifts the melting point notably less than does the higher shear (see Fig. 5).

RESULTS

The Effect of Shear on the Melting Point of Isolated DNA

This study was carried out on *E. coli* DNA of sedimentation constant 20, with a sharp melting point at 86°C (Fig. 1) in the solvent used throughout the work presented here: 0.02 M Tris-0.02 M EDTA-1% SDS. When the optical density (OD) was measured after cooling the heated solution to room temperature, the (non-ambient) melting point was 89.5°C (Fig. 1). The cooled solutions were also centrifuged in CsCl density gradients, and the percentage of the total DNA banding at the density of denatured DNA was measured in each case. These values were used

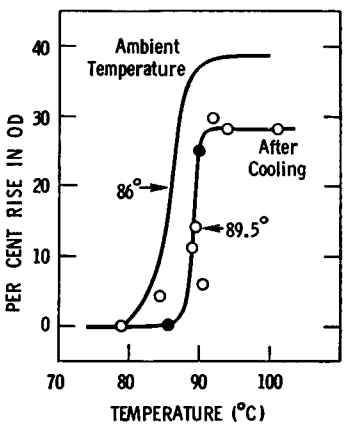

FIGURE 1 The melting point of isolated *E. coli* DNA in 0.02 M Tris-0.02 M EDTA-1% SDS, as shown by the rise in optical density. One curve was measured at the ambient temperature, and the other after cooling. The temperature at the midpoint of each curve is indicated in the figure. Filled symbols indicate that the sample was stirred while heating.

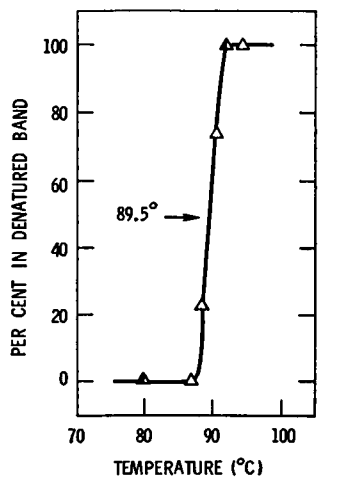

FIGURE 2 The melting of isolated *E. coli* DNA in 0.02 M Tris-0.02 M EDTA-1% SDS, as shown by the increase in density observed by centrifuging the cooled DNA in a CsCl gradient. Filled symbols indicate that the sample was stirred while heating.

to construct another nonambient melting curve, based on density (Fig. 2); this curve is coincident with the corresponding OD curve.

Stirring the DNA solutions at a very low shear rate with a magnetic bar (see Methods) during the heating period did not affect the melting curves (Figs. 1 and 2). However, the much higher shear rates obtainable in a glass homogenizer do shift the melting point downward, as shown in Table I. In this experiment the temperature was held at 80°C, where no denaturation can be demonstrated either without shear or with stirring (Figs. 1 and 2). Progressively higher shear rates produced increasing amounts of denaturation, as shown by banding in CsCl.

TABLE I

DENATURATION OF *E. COLI* DNA BY SHEAR AT 80°C*

Method of shearing	Shear rate	% denatured‡
	sec^{-1}	
Magnetic bar in test tube, 300 *rpm*	<630	0
Glass homogenizer, 400 *rpm*	3,500	15
Glass homogenizer, 1,500 *rpm*	13,000§	33

* DNA concentration: 0.01 mg/ml; solvent: 0.02 M Tris-0.02 M EDTA-1% SDS; time at 80°C: 20–25 min.

‡ Determined by banding in a CsCl density gradient.

§ Hershey et al. (1963) have shown that local denaturation can occur at sufficiently high stirring speeds in a blender.

The Effect of Shear on the Melting Point of DNA in E. coli Lysates

The melting curve of the DNA in an unfractionated *E. coli* lysate, containing Tris-EDTA-SDS solvent, is shown in Fig. 3 (curve 1). The cells were lysed at room

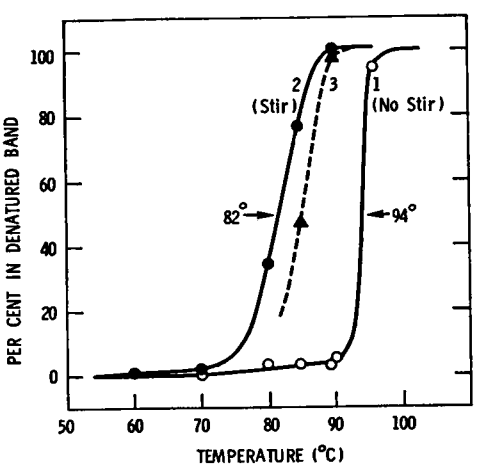

FIGURE 3 The melting of DNA in *E. coli* lysates. Cells were lysed in 0.02 M Tris-0.02 M EDTA-1% SDS at room temperature, without stirring. Curves 1 and 2: Aliquots of the lysates were then heated for 20 min at the indicated temperatures, with or without stirring. Curve 3: The lysate was stirred at room temperature first, then aliquots were stirred at the indicated temperatures. After heating, the samples were cooled and banded in a CsCl density gradient.

temperature, then heated as indicated, cooled, and banded in CsCl. The melting point is about 94°C, which is notably higher than the melting point of isolated DNA, 89.5°C (Fig. 2). Since the melting point of intracellular DNA (Fig. 4, discussed below) appears to be similar to that of isolated DNA, the additional stability in the lysate is probably an artifact, attributable to interaction of the DNA with divalent ions, polyamines, or other stabilizing elements from the cytoplasm.

When the lysate was stirred at a very low shear rate during the heating period, the melting point shifted downward 12 degrees, to 82°C (Fig. 3, curve 2). This confirms the effect of shear on the melting point of DNA. However, the DNA in the lysate is clearly much more sensitive to shear than the purified DNA in Figs. 1 and 2, whose melting point was unaffected by the same shear rate. The reason for this is undoubtedly the difference in viscosity. Although the DNA concentration is the same in both cases, the viscosity of the lysate is much greater, and its DNA is much

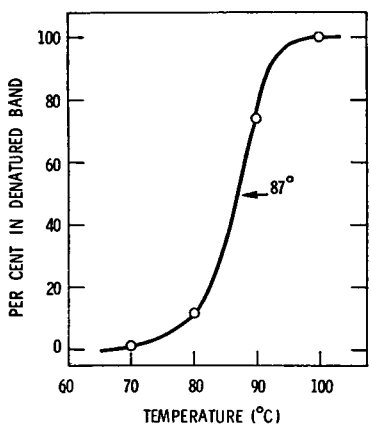

FIGURE 4 The melting of intracellular *E. coli* DNA. Cells were suspended in 0.02 M Tris, pH 8.0, heated for 20 min at the indicated temperature, then cooled, lysed, and banded in a CsCl density gradient. The temperature at the midpoint of the curve is approximately 87°C.

higher in molecular weight. Thus, shear *stress* (shear rate × viscosity)[1] is the parameter related to the DNA melting point. This was demonstrated by first stirring the lysate at room temperature, which partially degrades the DNA and lowers the viscosity; when the lysate was then stirred at higher temperatures, the shear stress was ower than before and the melting point was 85°C rather than 82°C (Fig. 2, curve 3).

The Effect of Shear on the Melting Point of DNA During Lysis of E. Coli

The melting curve of intracellular *E. coli* DNA is presented in Fig. 4. It was obtained by heating the cells in Tris, then lysing them at room temperature and banding the lysate in CsCl. The melting point, approximately 87°C, is slightly lower

[1] The viscosity required to calculate the pertinent shear stress is the microscopic "viscosity" of the DNA molecule itself, a quantity related to its molecular weight, not the macroscopic viscosity of the solution. We have shown this by varying the macroscopic viscosity without effect on the stirred melting point (unpublished data).

than that of isolated DNA in Tris-EDTA-SDS solvent (Fig. 2: 89.5°C), as would be expected, since the ionic strength of the latter solvent is slightly greater than the average intracellular ionic strength. This means that intracellular DNA is not notably stabilized or destabilized, although the broadness of its melting curve suggests some heterogeneity in that respect.

Lysis of *E. coli* at high temperatures, followed by cooling and banding in CsCl, produces the melting curve shown in Fig. 5 (curve 1). The melting point is 87°C. Stirring during lysis lowers the melting point to 83°C (Fig. 5, curve 2). Most significantly, however, the melti ng curve becomes biphasic, exhibiting a heterogeneously low-melting portion which accounts for about 25% of the DNA. The melting point of this component appears to be shifted still lower by higher shear rates (Fig. 5, curve 3). Furthermore, there is no low-melting component in vivo (Fig. 4),

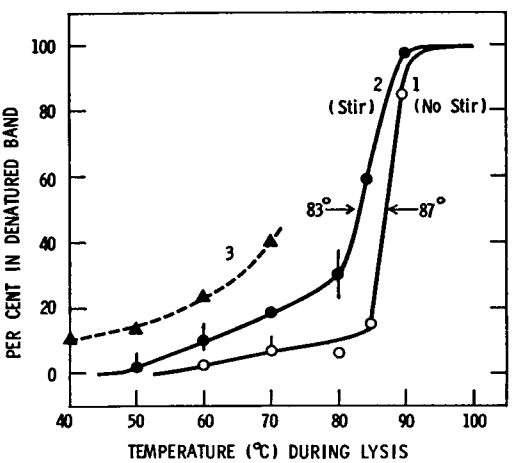

FIGURE 5 The melting of DNA during lysis of *E. coli*. Cells were lysed in Tris-EDTA-SDS at the indicated temperatures, with or without stirring. The dashed curve represents lysis in a concentric cylinder device at a shear rate of 630 sec^{-1}. The shear rate produced by stirring is much lower than this.

where there is no shear. We conclude that the low-melting material represents an especially shear-sensitive component of the DNA. Since this component is not present in the lysate, its unusual shear sensitivity must be eliminated when the cell is broken open and its contents diluted.[2]

Returning to the melting curve obtained during lysis *without* stirring (Fig. 5, curve 1), a small amount of low-melting material is present there also. This suggests that cell lysis itself subjects the DNA to a certain amount of shear.

There are two opposing factors which determine the melting point of the DNA: shear lowers the melting point, while the binding of ions, etc. tends to raise it; the increase in ionic strength contributed by the solvent also elevates the melting point. The balance of these factors is determined by whether the cells are stirred or not

[2] Complete loss of the extra shear sensitivity requires dilution as well as lysis. When cells are lysed with very little dilution of the cell contents, some of the DNA remains especially shear sensitive, although the amount of such DNA is reduced.

during lysis. Not only is the shear greater when stirring, but the factors that tend to raise the melting point also become somewhat more pronounced, since dilution of the cell contents is more rapid. These facts allow us to explain why the apparent lowering of the major melting point by stirring during lysis, from 87°C to 83°C (Fig. 5), is less than the melting-point lowering produced by stirring a lysate, from 94°C to 82°C (Fig. 3). The 87°C melting point during lysis without stirring obtains under conditions that are different from those of the other three melting points; if identical conditions were attainable, the true melting-point lowering of the main transition could be measured and would undoubtedly be the same for lysing cells as for a lysate.

DISCUSSION

Shear probably lowers the melting point of DNA by pulling the chains out of register in disordered regions, thereby promoting extension of the disarray rather than the restoration of order. Even a very low shear rate will produce a great deal of stress on the intact *E. coli* genome, when it is exposed at lysis, due to its great length. But it is still necessary to explain why a part of the *E. coli* genome is even more shear-sensitive than the major part of the DNA. This DNA melts normally within the cell, where none of the DNA is notably more or less stable to denaturation than isolated DNA. The especially shear-sensitive DNA might conceivably arise from a fraction of the cells which lyses differently from the rest, but this seems unlikely. More probably, it corresponds to the part of the genome most closely connected to the cell membrane or other large subcellular structures. At a given shear rate, the stress would be greatest on the DNA nearest the attachment point and might be expected to diminish with distance along the genome. This would produce the observed heterogeneity in the first part of the melting curve. Beyond a certain distance from the attachment point (which distance may well depend on the shear rate), the DNA would behave as though free of any foreign attachments and would therefore be subjected to lower and more uniform shear stress. This would produce the sharp main transition. After lysis the genome behaves as though it is no longer attached to any large structure; the melting curve in the lysate remains shear-dependent but contains only a single sharp transition.

Lysis itself appears to produce some shear. This lowers the melting point of the DNA somewhat so that, if lysis takes place at an elevated temperature, some of the especially shear-sensitive DNA may be denatured. The denaturation of the low molecular-weight newly-synthesized DNA of Okazaki et al. is very likely to have resulted, in part at least, from the shear of lysis at 37°C. The slightly denatured DNA isolated by Kidson was probably also produced by shear during lysis. If so, then their results imply that unusual shear sensitivity must be a property of newly-synthesized DNA near the growing point. Our investigations of the location of unusual shear sensitivity along the genome will appear elsewhere (Rosenberg and Cavalieri, 1968 *b*).

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