The degradation of T7 DNA in converging flow

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ABSTRACT
The flow-induced degradation of T7 DNA (Molecular Size = 40 Kbp) was studied in a flow device that generates converging flow rather than simple shear flow. We discovered that the sizes of the degradation products were very broadly distributed, covering the range from 10 Kbp to 36 Kbp. An explanation for the broadness of the distribution is given based on a computer simulation of the experiment. The significance of converging flow to the routine handling of large DNA is emphasized.

INTRODUCTION
We would like to bring to the attention of the reader recent results (1) on the degradation of T7 DNA in converging flow. Due to the early work of Hershey and Burgi on the degradation of T2 DNA in a blender (rotating stirrer) (2), it is generally believed that large DNA will align with the flow and break neatly in the middle. Although the flow field in a blender is not well known, subsequent experiments with simple shear flows seemed to produce similar degradation results (3, 4). Another type of flow which can lead to degradation is converging flow (5). Simple shear flow and converging flow are similar in the sense that they both generate forces that tend to stretch DNA. However, in simple shear flow there is a component that causes the DNA to rotate. When the molecule rotates it undergoes extension when oriented 45 degrees to the direction of flow and compression when oriented 135 degrees. Pure extensional flow, on the other hand, has no rotational component; therefore, the DNA is constantly under tension (6). Converging flow, which is a form of extensional flow and is referred to hereafter, occurs whenever liquid is forced through an orifice or a contraction. Degradation takes place upstream of the orifice where the extensional forces are greatest. Because converging flows are easily generated in common laboratory equipment, such as pipettes and hypodermic syringes, knowledge of the effect of this type of flow will be important to those who work with large DNA. In this article we show that, unlike shear flow, converging flow leads to a broad distribution of scission products rather than a narrow distribution.

METHODS
The flow cell where the degradation occurs consists of a plate with a 0.13 mm diameter orifice. Solutions of T7 DNA, concentration 10 µg/ml in BPES buffer (0.006 M Na₂HPO₄, 0.002 M Na₂H₂PO₄, 0.001 M Na₂EDTA, 0.179 M NaCl, pH=6.8), were continuously recirculated through the orifice by a peristaltic pump. (Control experiments in which the DNA solution was recirculated through the flow cell without the orifice plate showed no signs of degradation.) After a fixed period of recirculation at the desired flow rate, the degradation products were separated on a horizontal agarose gel electrophoresis unit, stained with ethidium bromide, and photographed. The analysis required that the photographic negative be scanned with a densitometer and that the digitized densitometer tracing be transformed to account for the exponential relationship between the film density and the amount of DNA. This transformation and further experimental details are given in reference 1.

RESULTS
Figure 1 shows the distribution of scission products after 20 minutes of recirculation at a flow rate of 2.65 ml/min. The small peak on the right is intact, native T7 DNA and represents 8% of the starting sample which was entirely intact T7 DNA. For this particular experiment 20 minutes of recirculation is equivalent to whom correspondence should be addressed
to each DNA molecule making approximately five passes through the orifice. The large asymmetric peak on the left, representing degraded T7 DNA, extends over a range from 10 Kbp to 36 Kbp. (The asymmetric appearance of the peak is due to the lack of sensitivity at the low size range; since there are degradation products in the range 30 Kbp to 36 Kbp, the complementary fragments in the 4.0 Kbp to 10 Kbp range, apparently are not being detected because of the limited sensitivity of the film.) When the recirculation is extended to longer times the peak corresponding to intact T7 DNA disappears. Shorter recirculation times give greater amounts of intact DNA and lesser amounts of scission products.

DISCUSSION

In one of the first theoretical papers on the mechanical degradation of polymers, Frenkel (7) showed that a flowing polymer solution will generate forces that tend to unravel the polymer from its random-coil state and align it in the direction of flow. Furthermore, he showed that high flow rates will not only align the polymer but also cause scission at the center of the molecule. An early experiment that confirmed this result was conducted by Hershey and Burgi on T2 DNA (2). They found that degradation in a blender could be controlled by adjusting the speed so that the scission products were halves or, at higher speeds, quarter-length molecules. Although the flow field in the blender is unknown, Hershey and Burgi’s results gave credence to Frenkel’s prediction that the polymer would align with the flow and break in the center.

The results of our experiments show, to the contrary, that the scission products are very broadly distributed covering nearly the entire length of the molecule. To help us understand the results, we ran a computer simulation which modeled the DNA as a series of beads connected by springs. The details of the simulation can be found in reference 1; however, we would like to describe the kinematics of the flow field because it is a key point to this experiment. In a converging flow device, such as the one used in our experiment, the liquid speeds up as it approaches the orifice. To a first approximation, the velocity increases as 1/r² where r is the radial distance from the orifice. As a consequence the time that the DNA spends in the fast flow is very short, while the extension rate, which changes as 1/r³, reaches very high values.

The simulation showed that the initial form of the chain, a random coil, became extended in the flow direction and compressed in the perpendicular directions, but the residence time in the fast flow was too short for the chain to lose all of its initial folds. Thus the stress maxima developed in the middle of the elongated sections between folds, wherever they happened to be, and the breaks that resulted from the stress maxima were distributed throughout the chain. While breaks were thus distributed, they were not uniformly distributed. This result is in agreement with the experiments of Figure 1, which shows that the breaks are most common near the middle, but not to the exclusion of breaks elsewhere.

As a practical matter many laboratory devices used to handle liquids can generate converging flows. Some examples include pipettes, hypodermic syringes and reduction fittings. The first study of shear-induced DNA breakage of which we are aware was made by Davison (8) using a syringe. Typical extension rates in these devices can vary quite widely depending on the flow rate and the size of the orifice. For example, the extension rates in the yellow and blue tips used with Gilson Pipetmans are typically in the range of 20 s⁻¹ to 100 s⁻¹ when the liquid is carefully expelled in drops rather than as a stream. In contrast, at a flow rate of 0.5 ml/s, the extension rate in a 1 ml syringe fitted with a 25 gauge needle is 318,000 s⁻¹. The same syringe without a needle and allowed to drain under the force of gravity has an extension rate of 750 s⁻¹. By comparison, the extension rates in our flow cell ranged from 4.7×10⁴ s⁻¹ to 7.8×10⁴ s⁻¹. At the lower extension rates no noticeable degradation of T7 DNA occurred until the sample made more than 70 passes through the flow cell. However, at the higher extension rates significant degradation was observed after two or three passes. Thus, large DNA, at least the size of T7 DNA, can be routinely handled without degradation providing high flow rates through small orifices are avoided.

REFERENCES