A ROTOR SPEED DEPENDENT CROSSOVER IN SEDIMENTATION VELOCITIES OF DNA'S OF DIFFERENT SIZES

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SUMMARY

There is an inverse relationship between the rotor speed and the sedimentation velocity for very high molecular weight DNA's. From hydrodynamic theory it has been predicted that a lower molecular weight DNA may sediment faster than a high molecular weight DNA at high rotor speeds. In this communication we provide experimental verification of this prediction, and we show that a crossover in sedimentation rates occurs as the rotor speed is raised.

INTRODUCTION

For very high molecular weight DNA, $M > 10^{+8}$, the sedimentation coefficient has been observed to be a function of the rotor speed[1-5]. Equations describing this phenomenon have been derived by Zimm[6] and are plotted in Figure 1. It may be seen that these theoretical curves cross each other. Thus, at low rotor speeds the higher molecular weight DNA has the greater sedimentation coefficient, while at high rotor speeds the converse is true.

We shall now show that this crossover may be demonstrated experimentally when mixtures of SP3 viral DNA, $s^{o}_{20,\omega} = 72S$, and *E. coli* or *B. subtilis* bacterial DNA's of $s^{o}_{20,\omega} \approx 100S$, are studied at different rotor speeds.

MATERIALS AND METHODS

E. coli strain TAU-bar was grown in a glucose-ammonium medium[7] supplemented with 2.5 μg/ml of uracil, and 200 μg/ml of Difco Bacto-peptone.

(Methyl-^{3}H)-thymidine (0.1 mC) of a specific activity of 3C/mmole obtained

from Schwarz Bioresearch was added to 4 ml of supplemented media with enough cold thymidine to give a final concentration of 4 μg/ml. Cells were grown into stationary phase at a concentration of 6 X 10^8 cells/ml. The cells were then filtered and washed on HA millipore filters, 0.45μ, and finally resuspended in 4 ml of N.E.T. buffer (0.15M NaCl, 0.01M Na EDTA, 0.01 tris adjusted to pH 8.3). B. subtilis MY2Y1U2 was grown in the same medium as the E. coli TAU-bar except that 2.5 μg/ml of L-leucine were also added.

SP3 phage from B. subtilis were grown as described by Romig[8], except that 1 mC of inorganic ^{32}P, carrier free, was added to 10 ml of Trypton broth half an hour before the bacterial culture was infected with the SP3 phage. The phage were purified by differential centrifugation followed by banding in CsCl.

Resuspended E. coli cells were gently mixed with a solution of 1% sodium dodecyl sulfate in a dialysis sac. SP3 phage labeled with ^{32}P were also added to give a concentration of phage DNA of about 0.2 μg/ml. Most of the SDS could be removed by dialysis against N.E.T. overnight. The pronase (claimed to be "free of nucleases"), B grade, Calbiochem, was heated at 80°C for 20 seconds, then added to a final concentration of 0.1 mg/ml. Half-a-milliliter of bacterial lysate was layered on top of a 5-20% sucrose-N.E.T. gradient using an Accropet (Manostat Corporation) with a "wide mouth" pipette, 2 mm I.D. All the centrifugation experiments were carried out at 20°C, with a SW 41 Ti rotor, in the L-2 preparatory centrifuge (Beckman).

THEORY

The equation derived by Zimm[6] may be written:

$$s^{\omega}/s^{o} = 1 - C_1U + C_2U^2 \ldots . \qquad (1)$$

The first 50 terms of this infinite series have been solved using

a digital computer[9]. In a purely empirical fashion it is found that Eq. 1 is closely approximated by the quadric

$$s^o/s^\omega = (1 + 0.924U)^{1/4} \qquad (2)$$

The symbols employed are:

$4C_1$ = 0.924 (for double stranded DNA)

$U = 5.97 \times 10^{-44} F^4/T\eta\nu^2$

$F = M(1 - \bar{v}\rho)\omega^2 r$

$\nu = s^o\omega^2 r$

s^o = sedimentation coefficient at zero rotor speed (sec^{-1})

s^ω = sedimentation coefficient at $\omega^2 r$ (sec^{-1})

ω = angular velocity (radians/sec)

r = radial distance (cm)

M = DNA molecular weight (daltons)

$\bar{v}$ = DNA partial specific volume (cc/g)

η = solvent density (g/cc)

T = absolute temperature (^oK)

To develop the theoretical curves shown in Fig. 1 we used the expression $s^o_{20,\omega}/\ s^o_{20,\omega,T2} = (M/M_{T2})^{0.35}$ of Burgi and Hershey[10] with $s^o_{20,\omega,T2}$ = 57S and $M_{T2} = 1.2 \times 10^8$ for calculating sedimentation coefficients at zero rotor speed. These values were then adjusted for the viscosity and density of a 10% sucrose solvent at 0°C assumed to be present in the simulated experiment. The radius was taken as 9.23 cm.

RESULTS AND DISCUSSION

The crossover in sedimentation coefficients which occurs with mixtures of different sized DNA's is clearly seen in Figure 2a,b. In these experiments *E. coli* DNA or *B. subtilis* DNA of about 100S was mixed with 72S DNA from phage SP3. The bacterial DNA's are highly heterogeneous in molecular weights, but we estimate that most of the DNA in these samples

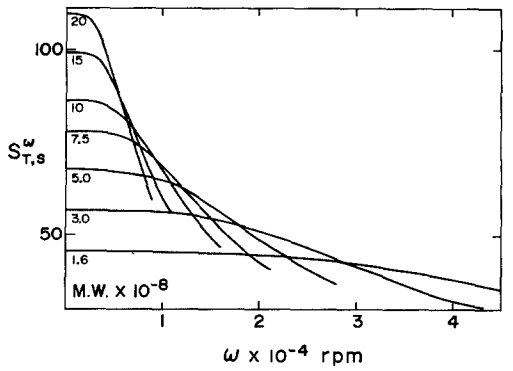

Fig. 1. A theoretical plot of s^{ω} versus ω for various sizes of DNA molecules. The sedimentation coefficients were calculated according to Equation 2 for 10% sucrose-N.E.T. buffers at 20°C. As indicated on the figure, the molecular weights range from 1.6 to 2.0 x 10^{+8} daltons.

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is between 3 and 5 million daltons. The SP3 DNA is about 1.6 x 10^{+8} daltons[11]. From Figure 1 it may be predicted that the crossover points should lie between 18,000 rpm and 29,000 rpm. From Figure 2a,b it may be seen that the crossover points are close to 19,000 rpm for the E. coli DNA and 29,000 rpm for the B. subtilis DNA.

As the molecular weight of the DNA being studied increases, the rotor speed effect should increase dramatically. The dominating term in Eq. 2 is M^4/s^{o2}. Since s^{o2} is approximately proportional to M, the overall molecular weight dependence is roughly to the third power. This means that as the molecular weight increases 15 fold in going from the large viral DNA's to the intact genome, the magnitude of the sedimentation effect will increase by 3400 fold. To offset this molecular weight dependence, the rotor speed may be reduced. The dependence on rotor speed is ω^4. Thus the speed reduction would be from 50,000 rpm to 6500 rpm.

The effect of rotor speed reduction on the sedimentation coefficient distribution of E. coli DNA is shown in Figure 3. The highest molecular weight species are retarded proportionately more, creating a false sharpening of the leading edge and a slow, narrow distribution. According to Eq. 2 the correct distribution is only obtained when the rotor

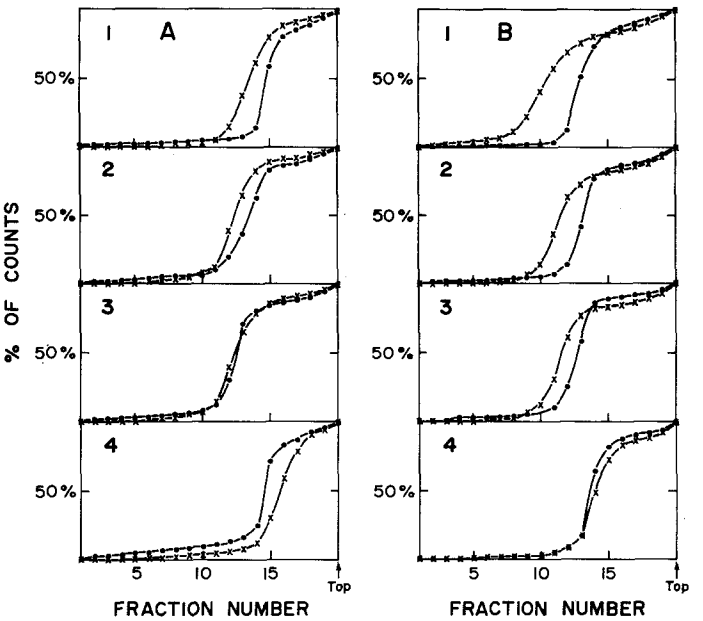

Fig. 2. A. A "crossover" series of sedimentation profiles for E. coli and SP3 DNA's at various rotor speeds. E. coli were labeled with ^{3}H (X - X) thymidine and SP3 (0 - 0) labeled with ^{32}P. A mixture of bacteria and phage were lysed in 4% SDS, then dialyzed and finally digested with 0.1 mg/ml pronase. Zone centrifugation was performed on a 5 to 20% sucrose-N.E.T. gradient using a SW 41 Ti rotor at (1) 9,600 rpm, (2) 14,500 rpm, (3) 19,000 rpm, and (4) 29,000 rpm. Approximately 50,000 counts/minute were recovered of ^{3}H(0.04 μg DNA) and 2000 counts/minute for ^{32}P(0.1μg DNA).

B. A "crossover" experiment using DNA's from ^{3}H labeled B. subtilis (X - X) and ^{32}P labeled SP3(0 - 0). (1) 9,000 rpm, (2) 14,000 rpm, (3) 18,500 rpm, (4) 29,000 rpm. Approximately 40,000 counts/minute were recovered for the ^{3}H(0.04μg DNA) and 2,000 counts/minute for the ^{32}P(0.1μgm DNA).

speed is dropped below 6500 rpm, as indicated in Figure 3 by the diamond-shaped experimental points.

The results presented in this communication serve as a qualitative confirmation of one of the predictions which may be made from an examination of Eq. 2, that is, the occurrence of crossover. This crossover occurs near the predicted rotor speeds, although heterogeneity in our bacterial DNA samples was too great to allow us to make a precise quantitative confirmation of Eq. 2. A more exact confirmation would be of importance, for

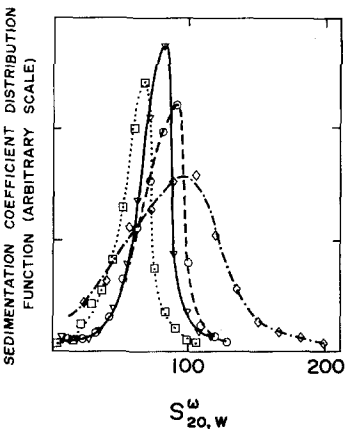

Fig. 3. Sedimentation coefficient distributions of E. coli DNA at various rotor speeds. The bacteria were labeled, lysed and centrifuged as described in Figure 2A, except that the rotor speeds employed were (◇— · —◇) 4,700 rpm, (0 - - - 0) 9,650 rpm, (○——○) 14,500 rpm and (□· · · □) 19,400 rpm.

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then it might be possible to use the speed at which crossover occurs as a measure of molecular weight for the largest DNA's.

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