Polydispersity and excluded volume effects in sheared DNA fragments

Dear Sir:

Two of us recently reported a variety of physical measurements on T2 bacteriophage DNA and its shear fragments (Harpst and Dawson, 1989). Also, we outlined an approach to estimating polydispersities of mechanically sheared samples by fractionation on methylated-albumin-kieselguhr (MAK) columns (Hershey and Burgi, 1960; Mandell and Hershey, 1960; Burgi and Hershey, 1961). In this communication, we provide a more quantitative treatment of the polydispersity of the DNA fragments. This, in turn, makes possible a more detailed analysis of excluded volume effects in these DNA's.

Many DNA samples used in current studies are subjected to shear breakage, and give DNA preparations which are heterogeneous in molecular weight (polydisperse). The degree of polydispersity influences the average values of physical properties of such samples. These effects were generally ignored in early studies, although various investigators, using a variety of approaches (Reinert, 1971; Reinert et al., 1971; Godfrey, 1976; Godfrey and Eisenberg, 1976; Dancis, 1978) showed that polydispersity must be considered in any quantitative correlation of properties with molecular weight. There are two alternatives for meeting this objective. One is to avoid the problem by using only monodisperse samples. For about two decades this has been possible because homogeneous DNA samples with a wide range of sizes are available, either from viruses or, more recently, from the use of restriction nucleases. However, in some investigations, particularly with synthetic polynucleotides, the samples are inevitably polydisperse, and one must include an analysis of the size distribution. To deal with synthetic materials, polymer chemists have developed several analytical treatments of size heterogeneity (Tung, 1967; Brandrup and Immergut, 1975; Kamide, 1977; Rabek, 1980). These make possible a quantitative treatment of the polydispersity of T2 DNA fragments used in our recent study (Harpst and Dawson, 1989).

A related issue involving these high-molecular-weight DNA's was raised in the previous study (Harpst and Dawson, 1989), where it was shown that significant contributions from excluded volume must be included to interpret light-scattering data. Specifically, the scaling, or excluded volume, exponent, ϵ, in $R^2 \sim N^{1+\epsilon}$, where R^2 is the mean-square radius, has a value $\epsilon \cong 0.08$. In this report, after applying polydispersity corrections to light-scattering, sedimentation, and viscometric data, we are able to estimate the excluded volume parameter,

$$z = \left(\frac{3}{2\pi \overline{L_0^2}}\right)^{3/2} \beta N_K^2, \qquad (1)$$

where $\overline{L_0^2} = N_K \ell_K^2$ is the unperturbed mean-square end-to-end distance, ℓ_K is the Kuhn statistical segment length, N_K is the number of Kuhn lengths in the chain, and β is the binary cluster integral (Yamakawa, 1971; Bloomfield et al., 1974).

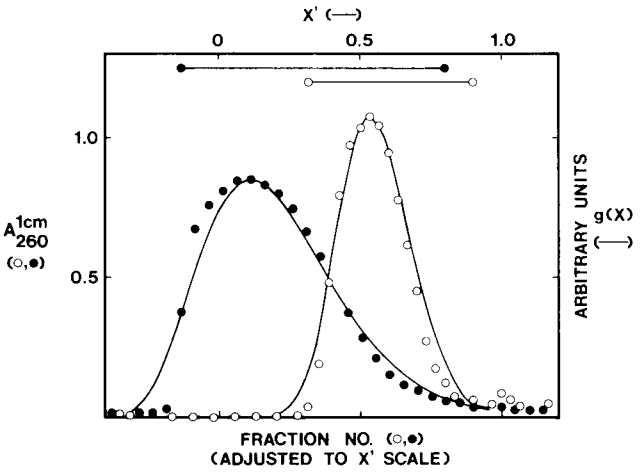

FIGURE 1 MAK column fractionations and length distributions of half and quarter fragments of T2 DNA. Open (○) and solid (●) circles indicate the elution profiles, A_{260}^{1cm} versus Fraction No., for halves and quarters, respectively. The solid curves (——) are the Schulz–Zimm distributions, $g(X)$ (Eq. 2), with the parameters in Table 1. X' is the molecular-weight scale given by Eqs. 5–8. The bars (○—○) designate pooled fractions in each sample.

EXPERIMENTAL PARAMETERS

The experimental data used here are from our earlier report (Harpst and Dawson, 1989). Whole T2 DNA is the full-length, native DNA, prepared from T2 bacteriophage. Half and quarter molecules of this T2 DNA were obtained by mechanical stirring. The polydispersity of sheared samples was defined by MAK column fractionation (Hershey and Burgi, 1960; Mandell and Hershey, 1960; Burgi and Hershey, 1961; Harpst and Dawson, 1989). The fractionation data for halves were corrected for a slight change in fraction volume on the high-molecular-weight side of the elution peak. This makes the elution profile slightly more symmetrical than that shown earlier (Fig. 1 of Harpst and Dawson, 1989). The elution profiles, normalized as described below, are given in Fig. 1.

It was shown previously (Harpst and Dawson, 1989) that the elution profiles (Fig. 1) can be fitted most accurately by a Schulz-Zimm weight distribution (S-Z) given by (Brandrup and Immergut, 1975; Kamide, 1977)

$$g(X) = \frac{y^{h+1}}{\Gamma(h+1)} X^h \exp(-yX), \qquad (2)$$

where

$$y = \frac{h}{\overline{X}_n}, \qquad (3)$$

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$$\frac{\overline{X}_w}{\overline{X}_n} = \frac{h+1}{h}, \quad (4)$$

$X = M/M_O$ is the degree of polymerization, where M and M_O are, respectively, the polymer and monomer molecular weights, $\overline{X}_w$ = weight-average X, $\overline{X}_n$ = number-average X, and $\Gamma(h+1)$ is the gamma function of $(h+1)$. To fit Eq. 2 to the experimental MAK column profiles, we assumed that fragment length was proportional to fraction number or volume, as suggested by earlier investigations (Hershey and Burgi, 1960; Burgi and Hershey, 1961). However, it is difficult to relate the variable X in Eq. 2 quantitatively to molecular weights of the samples, because the behavior of MAK columns in the low-molecular-weight range is poorly understood. Since the columns are eluted with salt gradients, each of which has a large, initial increase (≥ 0.4 M) in NaCl concentration (Hershey and Burgi, 1960; Mandell and Hershey, 1960; Burgi and Hershey, 1961; Sueoka and Cheng, 1967; Harpst and Dawson, 1989), the elution of low-M molecules must be markedly affected and, as a result, the position of zero molecular weight is ill-defined. For this reason, we must establish the true molecular-weight scale, X', where

$$M = X' M_{T2}, \quad (5)$$

M is any molecular weight (or average) at X', and M_{T2} is the known molecular weight of unbroken (monodisperse) T2 DNA.

To correlate the fitted S-Z variable, X, with the true length scale, X', we define the (unknown) origin, X_{min}, of $g(X)$ via the relationship

$$X = X' - X_{min} \quad (6)$$

and the equivalent form in terms of averages,

$$\overline{X}_w = \overline{X}'_w - X_{min}. \quad (7)$$

X' can be computed from two points on the experimental profiles. One is the small peak at high molecular weight, which is the elution position of whole T2 and is taken as $X' = 1$. The other well-defined point is the peak of the distribution curve for the fragments. Conveniently, the peak of the S-Z distribution is $\overline{X}_n$ (Tung, 1967). In our previous work, we assumed that $\overline{X}'_n = 0.5$ for halves and 0.25 for quarters, and obtained the corresponding fit of Eq. 2 to the experimental profiles (Harpst and Dawson, 1989). A more rigorous approach is to require the distribution to match the experimentally measured weight-average molecular weight ($\overline{M}_w$).

To accomplish this, we first translate the S-Z distribution obtained previously (Harpst and Dawson, 1989) to the experimentally determined value,

$$\overline{X}'_w = \frac{\overline{M}_w \text{ (halves)}}{M_{T2}}, \quad (8)$$

from Eq. 5 and the values in Table 1. From Eqs. 6 and 7, the parameters of the fitted S-Z distribution, and the ratios $\overline{X}'_w/\overline{X}'_n$ and $\overline{X}_w/\overline{X}_n$, a new value of X_{min} is calculated so that $\overline{X}'_w$ for the distribution coincides with the experimental $\overline{X}'_w$ from Eq. 8. The length scale, X', can then be adjusted to fit the fraction number of the experimental curve by assuming a linear relation between the two points corresponding to $X' = 1$, and the peak of the fragments, $\overline{X}'_n$, as derived above. Because translation of the initial distribution to new values of $\overline{X}'_w$ and $\overline{X}'_n$ alters the fit to the experimental profile, further iterations must be done by adjusting the parameters y, h, and X_{min}.

We show in Fig. 1 the resulting curves. Final parameters are included in the table. The results provide well-defined, analytical distributions for both the half and quarter fragments of T2 DNA.

ANALYSIS OF POLYDISPERSITY

Theory

Here we examine the effect polydispersity has on the measured properties, P_i. We assume a S-Z distribution and that the molecular-weight dependence has the general form

$$P_i = k_P X_i^{a_P}. \quad (9)$$

In Eq. 9 P_i is the property for a specie, i, of length X_i; k_P and a_P are constants for the given property. The properties of interest are: intrinsic viscosity, $[\eta]$, sedimentation coefficient, $s_{20,w}$, root-mean-square radius, R, and second virial coefficient, A_2.

On the basis of previous studies (Ford et al., 1973; McDonnell and Jamieson, 1976, 1977; Patterson and Jamieson, 1985; Shogren et al., 1986), we expect the z-average quantity, $\overline{R^2}_z$, determined from light-scattering (Tanford, 1961), to be most affected. For consistency, we define the property, P, as R; hence, the z-average is $\overline{P^2}_z$. Eq. 9 can be altered to give P_i^2 by replacing a_P with the term, $2a_P$.

Unfortunately, the standard relationships (Patterson and Jamieson, 1985) cannot be applied directly to our DNA data, because of the transposition of $g(X)$ to the X' scale (Eq. 6). To use the S-Z fits in Fig. 1, we derive equivalent expressions by evaluating the terms in $(X' - X_{min})^{a_P}$. (See, for example, Eq. 26 of McDonnell and Jamieson, 1976.) Appropriate results for the z-average quantities are obtained via the approach developed earlier (Ford et al., 1973; McDonnell and Jamieson, 1976; Patterson and Jamieson, 1985). By factoring out $\overline{X}'_w$, combining Eqs. 3, 4, and 7 with standard relations derived elsewhere (Patterson and Jamieson, 1985), and rearranging, we obtain

$$(P'_{m,w})^2 = \frac{\overline{P^2}_z}{\mathcal{Y}'(h, 2a_P, y, X_{min})}. \quad (10)$$

Here $P'_{m,w} = k_P(\overline{X}'_w)^{a_P}$ refers to a monodisperse solute with length = $\overline{X}'_w$, and $\overline{P^2}_z$ is the z-average mean-square value for the distribution, $g(X)$, on the length scale, X. The denominator in Eq. 10 is

$$\mathcal{Y}'(h, 2a_P, y, X_{min}) = (h + 1 + y \cdot X_{min})^{-2a_P} \left[\frac{\Gamma(h + 2 + 2a_P)}{\Gamma(h+1)}\right], \quad (11)$$

TABLE 1 **Experimental results for T2 DNA and its fragments, and parameters for polydispersity corrections**

Parameter (Units)	T2 DNA		
	Wholes	Halves	Quarters
Experimental results*			
$\overline{M}_w \times 10^{-6}$	115 ± 12‡	64 ± 0.5	24 ± 0.9
R (*nm*)	1224 ± 120	870 ± 20	523 ± 10
a (*nm*)	50 ± 5	48 ± 5	50 ± 5
ϵ	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
$A_2 \times 10^4$ ($mol\ cm^3 g^{-2}$)	2.0 ± 0.4	2.5	3 ± 1
$[\eta]$ (dl/g)	277 ± 5	182	113
$s_{20,w}$ (S)	63.0 ± 0.8	44.0 ± 1.3	31.3
a_R	—	0.57_5	—
a_η	—	0.56_7	—
a_s	—	0.45_2	—
Variables for polydispersity corrections with $g(X)$ in Fig. 1			
h		33	6
y		44.0	10.6
X_{min}		-0.21_6	-0.45_2
$\overline{X}'_w$ (expt'l)		0.55_7	0.20_9
$\overline{X}'_n$		0.53_4	0.11_4
PI'		1.04	1.83
Properties corrected for polydispersity			
$R_{m,w}$ (*nm*)		851	475
$[\eta]_{m,w}$ (dl/g)		183	115
$s_{m,w}$ (S)		44.2	31.8

Symbols and additional details are given in the text.
*All results are from Harpst and Dawson (1989).
‡Uncertainties shown are average deviations observed in original measurements or estimated errors in fitted parameters.

where all the parameters have been defined above. For the root-mean-square property, Eq. 10 becomes

$$P'_{m,w} = \frac{(\overline{P_z^2})^{1/2}}{[\mathcal{Y}'(h, 2a_P, y, X_{min})]^{1/2}}. \quad (12)$$

The treatment of weight-average properties, $[\eta]$ and $s_{20,w}$, is again similar to that described previously (McDonnell and Jamieson, 1976, 1977; Patterson and Jamieson, 1985; Shogren et al., 1986), but also must include translation to the X' scale. The above approach leads to the following relations for weight-average properties,

$$P'_{m,w} = \frac{\overline{P}_w}{\chi'(h, a_P, y, X_{min})}, \quad (13)$$

where $P'_{m,w}$ is defined above, and $\overline{P}_w$ is the weight-average property for the S-Z distribution, $g(X)$, on the length scale, X. The term $\chi'(h, a_P, y, X_{min})$ is

$$\chi'(h, a_P, y, X_{min}) = (h + 1 + y \cdot X_{min})^{-a_P} \left[\frac{\Gamma(h + 1 + a_P)}{\Gamma(h + 1)}\right], \quad (14)$$

where all parameters have been described above. Eqs. 13 and 14 provide polydispersity corrections to weight-average properties of distributions shown in Fig. 1.

Other properties of interest (A_2; persistence length, a; excluded volume parameter, ϵ) are discussed below.

Corrections to R, a, and ϵ

The magnitude of the polydispersity correction to the experimental value of R at X'_w is estimated from Eqs. 11 and 12 with an interpolated value of $(\overline{R_z^2})^{1/2}_{(calc)}$ on the X scale (cf. Eq. 5), calculated at $\overline{X}_w$ from the linear log R–log M relation with exponent, a_R, shown in the table. This yields $R'_{m,w}$, the value corrected for polydispersity at $M = \overline{X}'_w$. Because $R'_{m,w}$ is derived from the linear log R–log M plot, which does not run exactly through the data points, we determine the appropriate polydispersity correction to experimental results (Table 1) from the ratio of $R'_{m,w}/R'_{(calc)}$, where $R'_{(calc)}$ is the value of R from the smoothed log–log relationship at $\overline{X}'_w$.

A frequently used indicator of polydispersity is the polydispersity index, $PI = \overline{X}_w/\overline{X}_n$, for a given distribution, $g(X)$. For the translated distributions in Fig. 1, the polydispersity index on the X' scale ($PI' = \overline{X}'_w/\overline{X}'_n$) can be obtained from Eqs. 4 and 7 and is included in the table. We expected from earlier work that polydispersity corrections based on Eqs. 10–12 would lead to a slightly larger decrease in R than that predicted previously with R itself treated as a z-average (Patterson and Jamieson, 1985). The correction for halves (Table 1; $PI' = 1.04$) is 2%; i.e., insignificant. The larger correction of 10% for quarters (Table 1; $PI' = 1.8$) is comparable to the experimental uncertainty and, therefore, significant. We note that the corrected values of log R versus log $\overline{M}_w$ for whole, half, and quarter molecules of T2 DNA show an improved linear relationship.

The polydispersity corrections for R can be used to estimate the attendant changes in a and ϵ. Basic relationships for long, wormlike-coil polymers (Bloomfield et al., 1974) and standard treatments of errors (Bevington, 1969), indicate that polydispersity should decrease a and increase ϵ by about the same amount of its effect on R (Sharp and Bloomfield, 1968; Harpst, 1980). Hence, the estimated polydispersity effects on a and ϵ (2% for halves; 10% for quarters) are within experimental error and rather close to our previous estimates (Harpst and Dawson, 1989).

Corrections to s and [η]

Corrections to the weight-average properties, [η] and $s_{20,w}$, were made with Eqs. 13 and 14, the S-Z distributions of Fig. 1, and parameters in Table 1. The exponents, a_P, were obtained from linear fits of log [η] or $s_{20,w}$ versus log $\overline{M}_w$ (Table 1). For half and quarter molecules the corrections were negligible, as shown in Table 1.

Corrections to A_2

In the previous paper (Harpst and Dawson, 1989) we estimated the effect of heterogeneity on values of A_2 with equations derived by Casassa (1962) on the basis of hard-sphere theory (Yamakawa, 1971). This approach, along with the revised value of $PI' = 1.8$ for quarters (Table 1), leads to a maximum decrease in the observed A_2 of 5.6%. Although this is nearly twice as large as the correction estimated earlier (Harpst and Dawson, 1989), it is still well within the experimental uncertainty in A_2 (Table 1).

Recently, Tanaka and Šolc (1982) have presented an alternative estimate of polydispersity corrections for polymers characterized by S-Z distributions. The corrections are expressed in terms of the excluded volume expansion parameter, z_w, for a monodisperse polymer with $M = \overline{M}_w$ for the polydisperse samples (Eq. 34 of Tanaka and Šolc, 1982), and the binary cluster integral, β (Eq. 1). We can determine β for our DNA samples from the relationship derived by Shogren et al. (1986), who used the Yamakawa–Tanaka equation (Yamakawa, 1971) for chain expansion,

$$\frac{R_{m,w}}{\sqrt{N_K}} = \frac{\ell_K}{\sqrt{6}}\left(1 + 0.21\sqrt{N_K}\beta/\ell_K^3 - \cdots\right). \qquad (15)$$

In Eq. 15 $R_{m,w}$ is the experimental R for each sample, corrected for polydispersity (Table 1); $\ell_K = 2a$ was defined above (Bloomfield et al., 1974; Tanaka and Šolc, 1982; Shogren et al., 1986); and the number of Kuhn segment lengths, $N_K = \overline{M}_w/(M/\mathscr{L})\ell_K$, where $M/\mathscr{L} = 1{,}950$ g mol^{-1} nm^{-1} for NaDNA (Kam et al., 1981). The slope of a plot of $R_{m,w}/\sqrt{N_K}$ versus $\sqrt{N_K}$ gives β. For the three samples in Table 1, N_K varies from 123 for quarters to 590 for whole T2 DNA, clearly in the range (> 100 Kuhn lengths) where the theory should be applicable (Manning, 1981). Conveniently, the three data points (Table 1) define a near-perfect straight line from which we obtain $\beta = 5.33 \times 10^{-17}$ cm^3. This value of β and the parameters in Table 1 give $z_w = 0.28$, 0.46, and 0.62 for quarter, half, and whole T2 DNA, respectively. From Fig. 8 of Tanaka and Šolc (1982) and the values of PI and PI' (Table 1), which specify the minimum and maximum polydispersity corrections, we find that A_2 for halves has a negligible correction. The maximum correction for quarters increases A_2 by only 1%, a clearly insignificant change.

The above two methods provide polydispersity corrections to A_2 which are small, different in magnitude, and in opposite directions. For the DNA samples used here, the interpenetration parameter, ψ (Yamakawa, 1971), is ≤ 0.12, rather far removed from the large excluded-volume limit ($z_w \to \infty$) where $\psi \cong 0.25 \pm 0.05$, and consistent with the comparatively small z_w values deduced from our data. Note that the Casassa hard-sphere approach is expected to be applicable only at $z_w > 0.7$ (Yamakawa, 1971), which is well above the value for whole T2 DNA. The Tanaka–Šolc (1982) approach is expected to be accurate in the range, $z_w = 0$–5. We also remark on the striking linearity of the plot of $R_{m,w}/\sqrt{N_K}$ versus $\sqrt{N_K}$. This is consistent with the linear perturbation theory which provides the various equations used to obtain β (Yamakawa, 1971; Shogren et al., 1986).

DISCUSSION

We have quantitatively analyzed polydispersity effects on the measured R, $s_{20,w}$, and [η] of sheared DNA samples. It is clear that the corrections are negligible, except for the root-mean-square radius, R, of the heterogeneous quarter fragments. The corrected parameters in Table 1 provide a homologous series of results, suitable for comparison with those from other monodisperse DNA's (Freifelder, 1970; Reinert et al., 1971; Godfrey and Eisenberg, 1976; Voordouw et al., 1978).

It was noted earlier that MAK columns were originally developed for fractionating DNA (Hershey and Burgi, 1960; Mandell and Hershey, 1960; Burgi and Hershey, 1961; Sueoka and Cheng, 1967), but the column behavior in the low-molecular-weight range was not established. Calibration of the columns used in this study has been accomplished by allowing the S-Z distribution to be translated according to Eq. 6. As illustrated in Fig. 1, this allows the profiles of halves and quarters to be compared on the M (or X') scale. However, it leads to negative values of X_{min} and, apparently, to negative M's in the profile for quarters. Initially, this result appears troublesome, but it is insignificant for two reasons. First, the use of a large, initial increase in salt concentration makes uncertain the resolution of low M molecules. Undoubtedly, this contributes to the relatively poor fit of the S-Z distribution to the column profile of quarters at low M (Fig. 1). Second, the polydispersity analyses presented above are primarily influenced by fragment sizes at the profile peaks (Fig. 1) and higher.

Although the half and quarter fragments were fractionated with different columns, salt gradients, and fraction volumes (Harpst and Dawson, 1989), adjustment of the profiles to the molecular-weight scale (X') allows a direct comparison of the two samples (Fig. 1). The profile for halves, with the peak at

$\overline{X}'_n = 0.53$, confirms the fact that half-molecules predominate in this sample. The nearly symmetrical profile and low polydispersity index ($PI' = 1.04$) indicate the preparation is relatively homogeneous (Godfrey and Eisenberg, 1976; Rabek, 1980). The elution pattern may be taken as an indication of column resolution. On the other hand, the distribution for quarters is quite broad, with the predominant species at $\overline{X}'_n = 0.11$, not at 0.25. This indicates the sample was sheared well beyond quarters, and includes a large number of near one-eighth fragments. On the X' scale, this distribution appears to contain half, quarter, and eighth fragments, a mixture which evokes the shear mechanism originally proposed by Burgi and Hershey (1961). On the basis of the analysis presented here, it appears that the MAK column can separate fragments over the size range studied and provides a more quantitative fractionation than previously suggested (Dancis, 1978).

The experimental value of the binary cluster integral, β, derived from Eq. 15 for our DNA samples, is much larger than that for many synthetic polymers (Tanaka and Šolc, 1982). A theoretical estimate of β may be obtained from the expression, derived by Odijk and Houwaart (1978),

$$\beta = 8\pi\kappa^{-1}a^2, \tag{16}$$

who assumed that electrostatic repulsion was the major determinant. The term, κ^{-1}, is the Debye screening length. If we take $\kappa^{-1} = 0.68$ nm for 0.2 M salt (Odijk, 1979; Manning, 1981) and $a = 50$ nm from Table 1, Eq. 16 gives $\beta = 4.27 \times 10^{-17}$ cm^3, remarkably close to the value derived above from Eq. 15. The results presented here reaffirm the work of others who have shown or assumed that excluded-volume theory, originally developed for uncharged polymers, is quantitatively applicable to high-molecular-weight polyelectrolytes (Yamakawa, 1971; Bloomfield et al., 1974; Odijk and Houwaart, 1978; Odijk, 1979; Manning, 1981; Kam et al., 1981).

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