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Ultrasonic Scission of Deoxyribonucleic Acid in Aqueous Solution III. The Solution Properties of Sonicated Low Molecular Weight Samples as Revealed by Light Scattering and Viscosity

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ABSTRACT: The light scattering and viscosity measurements were carried out at 25°C in 0.2 M NaCl solutions for 14 fractionated low molecular weight DNA (sDNA) samples, which were prepared by the precipitational fractionation of the sonicated calf thymus DNA fragments. The relationship between the intrinsic viscosity $[\eta]$ and the weight-average molecular weight $\bar{M}_w$ could be expressed with an empirical formula, $[\eta]=9.54\times10^{-6}\bar{M}_w^{1.35}$ in $cm^3\,g^{-1}$ for $1.2\times10^5\leq\bar{M}_w\leq3\times10^5$, but a sigmoidal change followed with an inflection region ($3\times10^5\leq\bar{M}_w\leq6\times10^5$). The z-averaged square radius of gyration $\langle S^2\rangle_z$ was also determined from the angular dependence of scattered light intensity for each sDNA sample. The dependence of $\langle S^2\rangle_z$ and $[\eta]$ on $\bar{M}_w$ was calculated theoretically at various values of the persistence length for the A- and B-DNA conformations. It was found that the effect of the polydispersity on $\langle S^2\rangle_z$ and $[\eta]$ is important, if a quantitative comparison is made between the observed and calculated quantities.

KEY WORDS Sonicated DNA / Molecular Weight / Light Scattering / Intrinsic Viscosity / Mean Radius of Gyration /

In the study of the hydrodynamic property of DNA in solution, the flexibility of the double-helical chain is the major factor which must always be considered. The flexibility is quantitatively expressed by the persistence length q.[1-3] In order to evaluate the q value accurately, the molecular weight dependence of the mean square radius of gyration $\langle S^2\rangle$ must be determined.[4] For this purpose, the light scattering and viscosity measurements must be carried out, but these measurements require a large quantity of each DNA sample of known molecular weight. In particular, the less flexible low molecular weight samples are prerequisite of determining the degree of flexibility.

The short, monodisperse DNA samples in small quantities are now available conveniently by fragmenting either high molecular weight plasmid[5] or λ-phage[6] DNA with restriction enzymes, but the use of these fragmented samples in large amounts for hydrodynamic studies is prohibitive. The only practical method for preparing desired lower molecular weight DNA samples is the ultrasonic scission of a large amount of a high molecular DNA sample in a single batch. Doty *et al.* first prepared small molecular weight samples for the light scattering measurement by sonicating a calf thymus DNA.[7] Eigner and Doty were able to establish the intrinsic viscosity $[\eta]$ *vs.* weight-average molecular weight $\bar{M}_w$ relationship[8]; however, the DNA samples were not fractionated and the lowest molecular weight was still as high as 3×10^5. Godfrey and Eisenberg were able to obtain narrowly poly-

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disperse fractions ($\bar{M}_w = (2.8—13) \times 10^5$) by fractionating sonicated samples by the density gradient centrifugation method.[9,10] Record *et al.* sonicated a calf thymus sample in the water-glycerol mixture in an attempt to obtain much lower molecular weight samples.[11] They were able to prepare a number of fractions of sDNA ($\bar{M}_w = (0.3—3) \times 10^5$) by the gel permeation chromatography. By this method, however, a large quantity of a desired molecular weight sample can hardly be prepared.

In previous papers,[12,13] we reported the appropriate conditions for the ultrasonic scission of calf thymus DNA in aqueous solutions. We devised a successive precipitational fractionation method with acetone as the precipitant. The yield of each fractionated sample was about 100 mg or more in a single operation, and the lowest molecular weight was about 8×10^4 or 121 base pairs. The amount is sufficient for the physicochemical studies such as light scattering and viscosity. In this work, we report the characterization of each sample by the light scattering and viscosity methods, in order to find out the $[\eta]$ *vs.* $\bar{M}_w$ relationship for low molecular weight sDNA preparations. This relationship is immensely useful to evaluate $\bar{M}_w$ from the $[\eta]$ value, which is determined by a routine measurement. Discussion will be given on the effect of the polydispersity of sonicated sDNA samples on theoretical values of $\langle S^2 \rangle$ and $[\eta]$.

EXPERIMENTAL

Materials

The sonication of a calf thymus DNA sample (Worthington Biochemical Corp., U.S.A.) and the subsequent fractionation of sonicated DNA (sDNA) samples were described in detail in previous two papers.[12,13] All other chemicals were of reagent grade.

Measurements

The light scattering measurement was performed at 25°C on a Union Giken Model LS-601 light scattering photometer with the vertically polarized incident beam from a He–Ne gas laser (6328 Å). The instrument was calibrated with benzene which was filtered through a 0.2 μm pore Teflon membrane (Millipore Co.) to remove dusts. A value of 11.84×10^{-6} cm was used for the Rayleigh ratio $R(90)$ of benzene at the scattering direction ($\theta = 90°$), while a value of 1.4977 was used for the refractive index of benzene.[14] The stock sDNA solution was usually prepared by dissolving the freeze-dried sample (*ca.* 0.27—2.86 mg cm^{-3}) in 0.2 M NaCl solution. This sDNA solution was dialyzed at 4°C for 48 h in a Visking cellulose tubing (diameter 1.9 cm) against the 0.2 M NaCl unbuffered solvent; totally 6 liters of solvent were changed. The dialyzed sDNA solution and the final batch of the dialyzate solvent were filtered through the 0.22 μm pore Type FM membrane filter (Fuji Photo Film Co.), which was cleaned by soaking in distilled water at 7°C and then by being filtered several times with the 0.2 M NaCl solvent prior to use. The Rayleigh ratio $R(\theta)$ of a filtered sDNA solution at a mass concentration c was measured from 30° to 150° at an interval of 10°. This solution was then diluted to the second concentration with the filtered dialyzate solvent and subjected to Rayleigh ratio measurements. The concentrations were usually diluted in five successions. A value of 0.164 cm^3 g^{-1} was used for $(\partial n/\partial c)$ at 6328 Å,[16] while a value of 1.33 was assigned to the refractive index of the solvent.[17] The concentration of sDNA in the final solution was determined photometrically with the molar absorption coefficient $\varepsilon(P)$ of 6400 dm^3 mol^{-1} cm^{-1} at 260 nm in terms of the phosphorus unit.[18] For the unhydrated, completely dried (not freeze-dried) DNA sample, the mass concentration of 1 mg cm^{-3} corresponds to 3.03 mM of phosphorus.

The intrinsic viscosity of sDNA solution in 0.2 M NaCl was measured at 25°C with a dilution-type multibulb Ubbelohde viscometer or a single-bulb type with a flow time of

100 s.[12] For the successive dilution inside the viscometer, a dialyzed 0.2 M NaCl solvent was used. The partial specific volume $\bar{v}_2$ of sDNA in 0.2 M NaCl was 0.526 $cm^3\,g^{-1}$.

Analysis of Observed Data

Light Scattering. The following expression was used to analyze the observed intensity of light scattered from the sDNA solution.[4,19]

$$\frac{Kc}{R(\theta)}=\frac{1}{\bar{M}_w P(\theta)}+2A_2c+\cdots \tag{1}$$

where c is the mass concentration of sDNA in $g\,cm^{-3}$, $R(\theta)$ is the excess Rayleigh ratio at an angle θ between the scattered and transmitted beams, $\bar{M}_w$ is the weight-average molecular weight, $P(\theta)$ is the particle scattering factor. A_2 is the second virial coefficient and K is the optical constant. With the refractive index of solvent n_0, the specific refractive index increment of the solution $(\partial n/\partial c)$, the wavelength of the incident light λ_0 *in vacuo*, and the Avogadro number N_A, K is expressed for the vertically polarized light as follows:

$$K=\frac{4\pi^2 {n_0}^2}{N_A {\lambda_0}^4}\left(\frac{\partial n}{\partial c}\right)^2 \tag{2}$$

The value of $\bar{M}_w$ may be obtained by extrapolating both c and θ to zero as

$$\left(\frac{Kc}{R(\theta)}\right)_{c\to 0,\theta\to 0}=\frac{1}{\bar{M}_w} \tag{3}$$

The value of $P(\theta)$ is dependent on the shape of solute and related to the z-averaged square radius of gyration $\langle S^2\rangle_z$ as[4]

$$P(\theta)^{-1}=1+\frac{16\pi^2}{3\lambda^2}\langle S^2\rangle_z \sin^2\left(\frac{\theta}{2}\right)+\cdots \tag{4}$$

where $\lambda=\lambda_0/n$, n being the refractive index of the medium. Hence, the value of $\langle S^2\rangle_z$ can be evaluated from the angular dependence of $(Kc/R(\theta))_{c\to 0}$. Kratky and Porod introduced the persistence length q to describe the flexibility of the polymer chain.[20] Sharp and Bloomfield have related $P(\theta)$ to q as[21]

$$P(\theta)=\frac{2}{x^2}(x-1+e^{-x})+\frac{4}{15L}+\frac{7}{15xL}-\frac{11}{15L}e^{-x}-\frac{7}{15xL}e^{-x} \tag{5}$$

where $x=(1/6)(8\pi q/\lambda)^2(\sin^2(\theta/2))L$, L is the reduced contour length $(=l/2q)$, and l is the contour length. Benoit and Doty have shown that[22]

$$S^2=\frac{ql}{3}-q^2+\frac{2q^3}{l}\left[1-\frac{q}{l}(1-e^{-l/q})\right] \tag{6}$$

Equation 6 reduces to $S^2=ql/3$ for $q\ll 1$ and $S^2=l^2/12$ for $q\gg l$.

Intrinsic Viscosity. The intrinsic viscosity $[\eta]$ may be expressed as

$$[\eta]=\bar{v}_2\times\nu(p) \tag{7}$$

where $\bar{v}_2$ is the specific volume of solute and $\nu(p)$ is the shape factor which is the function of the axial ratio p. For the rodlike, *i.e.*, cylindrical molecule, Simha has derived the following:[23,24]

$$\nu(p)=\frac{p^2}{15}\left(\frac{1}{\ln 2p-1.8}+\frac{3}{\ln 2p-0.8}\right)+\frac{14}{15} \tag{8}$$

while Yamakawa and Fujii have shown theoretically as[25,26]

$$\nu(p)=\frac{p^2}{6\left(\ln p+2\ln 2-\frac{7}{3}\right)} \tag{9}$$

(The original formula (eq 36 of ref 25) was recast by letting $\bar{v}_2=(\pi N_A/M_l)(a/2)^2$, where a is the diameter and M_l is the mass per unit length.) The latter authors have also derived the following expression for the flexible chain:[25]

$$[\eta]=\frac{\Phi(L,d)L^{3/2}(2q)^3}{M} \tag{10}$$

where d is the reduced diameter $(=a/2q)$ of the chain. Φ is the function of L and d, being 2.86×10^{24} for the random-coiled polymer chain. In eq 10, the specific volume $\bar{v}_2$ does not appear explicitly.

Table I. Hydrodynamic properties of fractionated sDNA in 0.2 M NaCl

Sample[a]	Chip[b]	p[c] / W	Fraction no.[d]	$\bar{M}_w$ / 10^4 g mol^{-1}	$\langle S^2\rangle_z$ / 10^2 nm^2	$[\eta]$ / 10^2 cm^3 g^{-1}	$\bar{M}_w/\bar{M}_n$[e]	H[f] / %
I	n	200	4	8.3	2.7	0.29	1.06	33.7
II	n	200	3	14.2	6.9	0.87	1.08	39.7
III	n	105	3	17.9	10.2	1.15	1.18	37.9
IV	n	200	2	24.6	16.1	1.65	1.29	39.0
V	s	25	3	37.0	30.4	2.17	—	—
VI	n	105	1	43.8	33.0	2.41	1.29	41.3
VII	n	60	2	70.3	51.1	2.86	1.44	—
VIII	s	60	1	85.8	67.1	3.35	—	—
IX	s	25	1	86.0	59.2	4.08	—	—
X	n	25	2	124	101	5.18	1.77	—
XI	n	200	1	30.3	30.0	2.24	—	41.7
XII	n	200	2	25.2	25.6	1.91	—	39.8
XIII	n	200	3	23.0	15.2	1.52	—	39.4
XIV	n	200	4	12.2	6.7	0.74	—	37.4

[a] Samples **XI**—**XIV** were newly prepared for the present work.
[b] The shape of the sonicator probe; standard (n) and small (s) chips.
[c] The nominal irradiation intensity in watts.
[d] The fraction number of the sample obtained by the precipitational fractionation of an irradiated original DNA preparation.
[e] These data were determined by the acrylamide gel electrophoretic method.[13]
[f] The hyperchromicity, defined as $H=[(A_d-A_n)/A_n]\times 100$, where A_n and A_d are the absorbances at 260 nm of an sDNA solution at 20°C and 95°C, respectively.[13]

RESULTS AND DISCUSSION

Light Scattering and the Zimm Plot

Figure 1 shows three examples of the Zimm plot for sDNA solutions. The $Kc/R(\theta)$ *vs.* $\sin^2(\theta/2)+kc$ plots are nearly linear in all cases. Extrapolations of $Kc/R(\theta)$ values to zero concentration and to zero angle are thus reliable, yielding both $\langle S^2\rangle_z$ and $\bar{M}_w$ reasonably well. Values of $\langle S^2\rangle_z$ and $\bar{M}_w$ are given in Table I.

Intrinsic Viscosity

Figure 2 shows examples of the variation of the reduced viscosity η_{sp}/c with the concentration c for fractionated sDNA solutions in 0.2 M NaCl. For the higher molecular weight samples, a quadratic dependent of η_{sp}/c becomes appreciable. The Huggins constant k_1 ranges between 0.2 and 1.4, a close agreement with the theoretically predicted values of 0.36—2.26.[27] The intrinsic viscosity of each sDNA sample is given in Table I.

Relationship between $[\eta]$ *and* $\bar{M}_w$

Figure 3 shows the relationship between the intrinsic viscosity $[\eta]$ and the weight-average molecular weight $\bar{M}_w$, obtained from the light scattering measurement, for 14 fractionated sDNA samples. The dependence of $[\eta]$ on $\bar{M}_w$ is surprisingly sigmoidal, indicating that the double-helical backbone of DNA transforms hydrodynamically from the nearly rodlike form ($8\times 10^4 < \bar{M}_w < 3\times 10^5$) to the wormlike, coiled form ($\bar{M}_w > 7\times 10^5$) with the increase in the molecular weight, hence, the chain length. The very gradual change of $[\eta]$ with $\bar{M}_w$ appears in the range of $\bar{M}_w$ between 3×10^5 and 7×10^5. In the possibly rodlike form region, the following empirical formula was obtained,

$$[\eta]=9.54\times 10^{-6}\,\bar{M}_w^{1.35} \qquad (11)$$

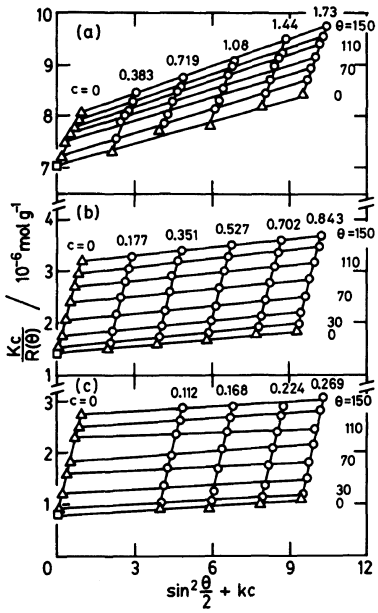

Figure 1. The Zimm plots of sDNA in 0.2 M NaCl. (a) Sample **II**; (b) Sample **VII**; (c) Sample **X**. The constants, k, in cm^3 mg^{-1} are 3.5 (a), 11 (b), and 35 (c). The numerals are c in mg cm^{-3} and θ in degrees. The optical constant K is 1.94×10^{-7} cm^2 mol^{-1} g^{-2}. Circles; observed points, triangles; extrapolated points, square; doubly extrapolated point.

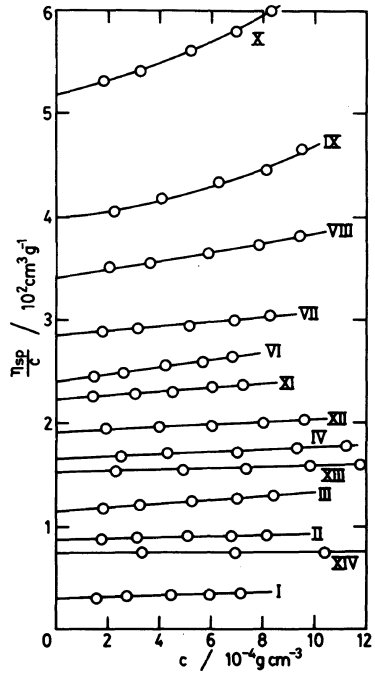

Figure 2. The reduced viscosity η_{sp}/c *vs.* the mass concentration c of sDNA in 0.2 M NaCl. The Roman letters stand for the sample number given in Table I.

for $1.2\times10^5 \leq \bar{M}_w \leq 3\times10^5$. Eigner and Doty proposed an empirical formula for a series of native, but unfractionated polydisperse DNA preparations of different sources:[8] $[\eta]=10.5\times10^{-6}\bar{M}_w^{1.32}$ for $3\times10^5\leq\bar{M}_w\leq2\times10^6$, according to which the dashed line is drawn in Figure 3. The slight difference in the factors between our (eq 11) and the Eigner and Doty formulas should be attributed to the fact that our formula is derived on the basis of observed values, whereas Eigner–Doty's is an extrapolation beyond experimental points.

Godfrey and Eisenberg have determined the $[\eta]$ *vs.* $\bar{M}_w$ relationship ($\bar{M}_w>3\times10^5$);[9,10] their observed points are shown with filled squares in Figure 3. Mandel and Schouten recently reported the $[\eta]$ *vs.* $\bar{M}_w$ relationship for sonicated short DNA preparations at various ionic strengths.[28] The open square in Figure 3 is their experimental point in 0.2 M NaCl. When $\bar{M}_w$ is less than *ca.* 4×10^5, all the observed points are rather close to each other, but the gradual transition between 3×10^5 and 7×10^5 in $\bar{M}_w$ was not detected by those previous workers. The exponent of 1.35 in eq 11 or 1.32 in the Eigner and Doty formula indicates that the backbone chain of sDNA at a high salt concentration of 0.2 M is not completely rigid but slightly flexible (for the rigid rod, the exponent should be 1.8[4]).

Molecular Conformations Revealed by Light Scattering Data

Figure 4 shows experimental relationship between $\langle S^2\rangle_z$ and $\bar{M}_w$ for sDNA solutions (*cf.* Table I). A sigmoidal change was also observed in Figure 4, as in Figure 3. It is possible to calculate theoretically the $\langle S^2\rangle_z$ value for a given q value with the aid of eq 6, provided that the contour length l is specified. The length l can be estimated from $\bar{M}_w$ value,

if the conformation of DNA is specified. (The mean weight of a base pair is 660 g mol^{-1} and, for example, the B-form DNA: $l = 3.4 \times \bar{M}_r/660$ Å). The observed mean-square radius of gyration is the z-averaged quantity, while the molecular weight is the weight-averaged quantity; hence, the molecular weight distribution or the polydispersity of a given sDNA sample should affect the $\langle S^2 \rangle_z$ *vs.* $\bar{M}_w$ relationship. For the sonicated and fractionated sDNA sample, the logarithmic-normal distribution[29] (the Wesslau distribution[30]) function is a good approximation to describe the molecular weight distribution.[13] Therefore, this function will be employed in the following discussion. The degree of the polydispersity of a given sample may be specified by the ratio of the weight-average molecular weight $\bar{M}_w$ to the number-average molecular weight M_n, *i.e.*, $\bar{M}_w/\bar{M}_n$.[13]

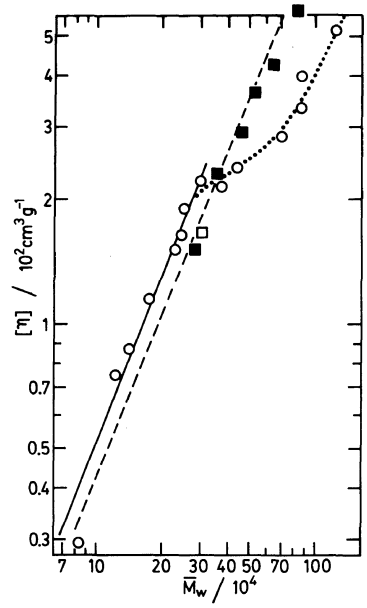

Figure 3. The relationship between the intrinsic viscosity $[\eta]$ and the weight-average molecular weight $\bar{M}_w$ of sDNA in 0.2 M NaCl. Circles are determined in the present work, filled and open squares are the data taken from ref 9 and 28, respectively. The solid line is given by $[\eta] = 9.54 \times 10^{-6}\ \bar{M}_w^{1.35}$. The dashed line is given by the Eigner–Doty formula.[8]

Figure 4 shows the theoretical $\langle S^2 \rangle_z$ *vs.* $\bar{M}_w$ plots for both A- and B-form conformations with different $\bar{M}_w/\bar{M}_n$ values ($\bar{M}_w/\bar{M}_n = 1$ for the monodisperse system). In each figure the persistence length q was assumed to be ∞ (rigid rod), 550, 350, and 250 Å with the increase in the backbone flexibility. A comparison of these theoretical curves with the experimentally evaluated $\langle S^2 \rangle_z$ *vs.* $\bar{M}_w$ relationship reveals several interesting points. First, the monodisperse assumption results in a very poor agreement; hence, the molecular weight distribution must always be taken into account, unless the DNA sample is *truly mono-*

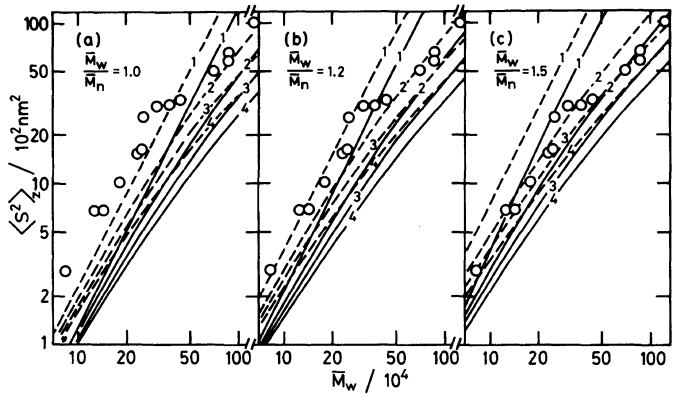

Figure 4. The dependence of the z-average square radius of gyration $\langle S^2 \rangle_z$ on the weight-average molecular weight $\bar{M}_w$. The solid lines are theoretical curves calculated for the A-form DNA, while the dashed lines are for the B-form DNA. Circles are experimental points. The numerals represent the persistence length q in Å; ∞ for 1, 550 for 2, 350 for 3, and 250 for 4. For other details, see the text.

disperse. Secondly, the persistence length is large only in the limit of low molecular weight, remaining up to an $\bar{M}_w$ of 2×10^5 and then it appears to transfer rather smoothly to a smaller value at higher $\bar{M}_w$'s; this change may be explained as a transformation of the linear double helical structure to a super helical coil.[31] Thirdly, the exact conformation of DNA (the A- *vs.* B-form) is rather difficult to determine from the light scattering data alone (*cf.* Figures 4a—c), because the contour length l must be assumed for evaluating q values (eq 5); this limits the possible solution conformation to the crystallographically known (*e.g.*, the A- *vs.* B-form) structures. Fourthly, the polydispersity is the critical factor to evaluating the persistence length for slightly flexible DNA chains; this is the point that should not be overlooked. The degree of polydispersity was estimated to be in the range of 1.1—1.5 in terms of $\bar{M}_w/\bar{M}_n$ for the present fractionated sDNA samples from the polyacrylamide gel electrophoretic measurement.[13] The theoretical $\langle S^2\rangle_z$ *vs.* $\bar{M}_w$ plots with $\bar{M}_w/\bar{M}_n$ values of 1.2—1.5 apparently satisfy the experimental data in Figures 4a—c. Hence, the overall conformation of short DNA fragments may be closer to the B-form than to the A-form, with q-values of 550—350 Å in a high salt concentration of 0.2 M NaCl.

In two extreme cases, where the polymer chain is either rigid rodlike or random-coiled, $\langle S^2\rangle_z$ is given from eq 6 as

$$\langle S^2\rangle_z=\frac{1}{12}\left(\frac{\bar{M}_w}{\bar{M}_l}\right)^2\left(\frac{\bar{M}_{z+1}\bar{M}_z}{\bar{M}_w{}^2}\right) \quad \text{for rigid rod} \qquad (12)$$

$$\langle S^2\rangle_z=\frac{q}{3}\left(\frac{\bar{M}_w}{\bar{M}_l}\right)\left(\frac{\bar{M}_z}{\bar{M}_w}\right) \quad \text{for coil} \qquad (13)$$

Letting $V_1=(\bar{M}_{z+1}\bar{M}_z)/(\bar{M}_w{}^2)$ and $V_2=\bar{M}_z/\bar{M}_w$, $V_1=V_2=1$ for the monodisperse system. For the logarithmic-normal distribution function, $V_1=\exp(3\omega^2/2)$ and $V_2=\exp(\omega^2/2)$, where $\omega(>0)$ is a parameter for the breadth of the distribution.[30] For a given degree of polydispersity, *i.e.*, $\omega>0$ and $\bar{M}_w/\bar{M}_n>1$, the $\langle S^2\rangle_z$ value is more affected by the polydisperse solutes in the rodlike state than in the random-coiled state. For example, if $\bar{M}_w/\bar{M}_n=\exp(\omega^2/2)=1.5$, then $V_1=3.38$ and $V_2=1.5$. This suggests that the value of $\langle S^2\rangle_z$ is large by 3.38 for the rod and by 1.5 for the coil, as compared with the mean-square radius of gyration for the monodisperse system ($\bar{M}_w/\bar{M}_n=1$). It should be noted that the effect of polydispersity is critical in dealing with the radius of gyration or the persistence length of the rodlike chain conformation.

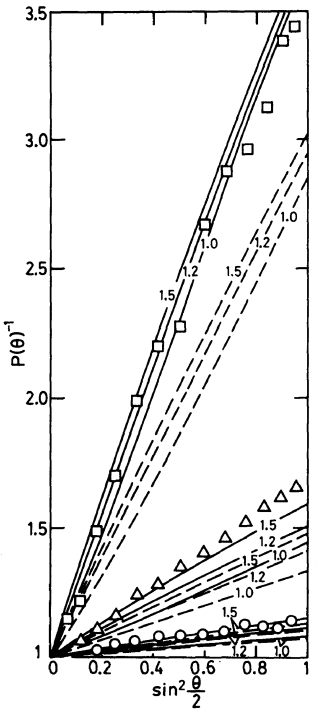

Figure 5. The dependence of the reciprocal of the particle scattering factor $P(\theta)^{-1}$ on the angle between transmitted and scattered light beams, $\sin^2(\theta/2)$. Symbols are experimental points for three samples with $\bar{M}_w/10^4$ of 124 (□), 37.0 (△), and 14.2 (○). The solid and dashed lines are theoretical curves. The persistence lengths are assumed to be 550 Å (——) and 350 Å (------). The numerals stand for the degree of polydispersity $\bar{M}_w/\bar{M}_n$, as specified.

Angular Dependence of Scattering Factor

Figure 5 shows the angular dependence of

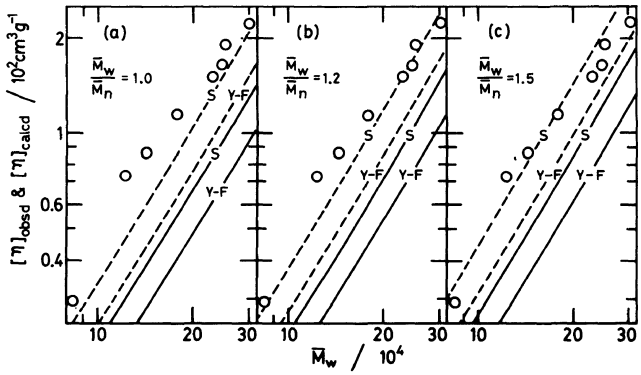

Figure 6. The dependence of the intrinsic viscosity on the weight-average molecular weight of sDNA samples in 0.2 M NaCl. Circles are experimental points. The solid and dashed lines are theoretical curves. The conformations of DNA are assumed to be the A-form (——) and the B-form (------). "S" stands for the Simha equation, while "Y-F" stands for the Yamakawa-Fujii equation for the rodlike polymer chain.

the reciprocal of the scattering factor $P(\theta)^{-1}$ for three sDNA samples. Observed $P(\theta)^{-1}$ values (symbols) are compared with the theoretical curves, which were calculated with the expression given by Sharp and Bloomfield (eq 5) and also with the numerical tables given by Yamakawa and Fujii.[32] Values of 550 and 350 Å were employed for the parameter q and the distribution function and the degree of polydispersity were assumed to be the same as those in the above section. For the short chain DNA with an $\bar{M}_w$ of 14.2×10^4 (circles) experimental $P(\theta)^{-1}$ *vs.* $\sin^2(\theta/2)$ plot agrees with the theoretical curves specified by $\bar{M}_w/\bar{M}_n = 1.5$ and $q = 550$—350 Å, thus indicating that the degree of polydispersity is the more important factor for the angular dependence of $P(\theta)^{-1}$.

For the medium size DNA ($\bar{M}_w = 3.7 \times 10^5$), both $\bar{M}_w/\bar{M}_n$ and q values are rather critical for a good fitting (triangles). As the molecular weight increases ($\bar{M}_w = 124 \times 10^4$), $P(\theta)^{-1}$ values become quite sensitive to q values but rather insensitive to $\bar{M}_w/\bar{M}_n$ (squares). Hence, it may be concluded that the particle scattering factor is affected mostly by the persistence length, but not much by the degree of polydispersity, for higher molecular weight flexible samples; reverse is the case for the smaller samples.

Molecular Conformations Revealed by Intrinsic Viscosity

In the lower molecular weight region ($\bar{M}_w < 3 \times 10^5$), the shape of sDNA may be approximated with a roughly cylindrical model. The experimental relationship in Figure 3 may be compared with the theoretical one, which may be calculated by using the Simha expression (eq 8) and also the Yamakawa and Fujii formula (eq 9) on the assumption similar to the $\langle S^2 \rangle_z$ *vs.* $\bar{M}_w$ plots in Figure 4. In calculating $[\eta]_{calcd}$ in eq 7, a value of 0.526 $cm^3 g^{-1}$ was used for $\bar{v}_2$ and the effect of polydispersity on $\nu(p)$ was taken into account (the Wesslau function, $\bar{M}_w/\bar{M}_n$, etc., as in the preceding sections[33,34]).

Figure 6 shows the theoretical curves, which can be compared with the experimental data (symbols). For a given molecular weight $\bar{M}_w$, the calculated intrinsic viscosity $[\eta]_{calcd}$ is larger for the B-form DNA, simply because the axial ratio, hence, the axial translation is larger (3.4 Å/base pair for B-form *vs.* 2.56 Å for A-form). The radius of the helix was assumed to be 20 Å for both conformations. The Yamakawa–Fujii equation (eq 9) yields much smaller intrinsic viscosity than the Simha equation (eq 8) for a given $\bar{M}_w/\bar{M}_n$, but the curvatures for the $[\eta]$ *vs.* $\bar{M}_w$ plots are always

very close to each other. This is because of the fact that a factor of 1/15 was used in the first term of $\nu(p)$ in eq 8 in accordance to the original Simha formula.[23,24] Some workers prefer another factor of 2/45,[4,35] which, however, yields 1.5-fold smaller $[\eta]_{calcd}$ values. The sDNA chains are slightly bent in 0.2 M NaCl, even if the molecular weight are less than 3×10^5. It is, therefore, understandable that the agreement is poor between the observed and calculated $[\eta]$ values, even if the degree of polydispersity is taken into account in eq 8 or 9. This discrepancy may be corrected by introducing another parameter of the hydrodynamic volume v_h,[4] which is, however, difficult to evaluate.

For the flexible backbone polymer, the theoretical expression (eq 10) of Yamakawa and Fujii may be applied to estimate the $[\eta]$ *vs.* $\bar{M}_w$ relationship. Figure 7 shows the observed and theoretical dependence of $[\eta]$ on $\bar{M}_w$, where the DNA sample is assumed to be monodisperse, because the effect of the degree of polydispersity $\bar{M}_w/\bar{M}_n$ cannot be estimated.[25] As in Figure 4, the persistence lengths are changed for the two DNA conformations. The agreement between observed and calculated $[\eta]$ values is good for the B-form DNA in (b). This may be fortuitous, since the molecular weight distribution is not taken into account to theoretically treat the intrinsic viscosity. The effect of the diameter of the polymer chain on the $[\eta]$ *vs.* $\bar{M}_w$ relationship is not too critical. Values of $[\eta]$ become smaller, as q values are smaller for a given molecular weight $\bar{M}_w$. If DNA is assumed to be in B-form, q is about 550 Å, but this value is not constant and seems to vary with $\bar{M}_w$. This result is in contrast with the observations by Godfrey and Eisenberg[9,10] and Yamakawa and Fujii.[25] It is interesting to note, however, that the theoretical expression, derived by Yamakawa and Yoshizaki on the basis of the helical wormlike chain model, shows an inflection on the $[\eta]$ *vs.* $\bar{M}_w$ plot.[31] An inflection found in both $[\eta]$ *vs.* $\bar{M}_w$ and $\langle S^2 \rangle_z$ *vs.* $\bar{M}_w$

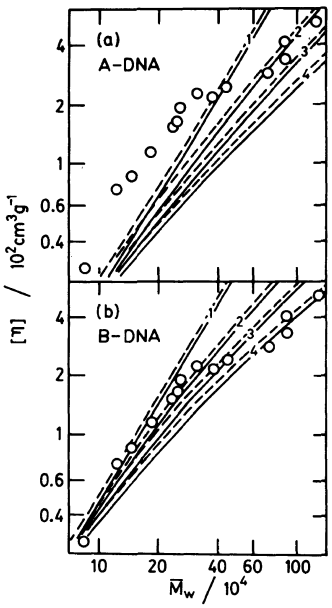

Figure 7. The dependence of the intrinsic viscosity on the weight-average molecular weight of sDNA samples in 0.2 M NaCl. Circles are experimental points. The solid and dashed lines are theoretical curves calculated for the A-DNA (a) and the B-DNA (b) with the Yamakawa–Fujii equation for the flexible polymer chain.[25] The persistence lengths are indicated by numerals: (1) ∞, (2) 550 Å, (3) 350 Å, and (4) 250 Å. The diameter of the polymer chain is 20 Å (——) and 26 Å (------).

plots (Figures 4 and 7) may be related to the higher order structure of DNA, such as super helix coil. This is certainly a future subject.

CONCLUDING REMARKS

It may now be concluded that the gross conformation of sDNA's with smaller molecular weights is perhaps close to the B-form in 0.2 M NaCl, as compared with the A-form. It is, however, difficult to identify the exact conformation by the light scattering (*cf.* Figure 4) and the intrinsic viscosity (*cf.* Figure 6) alone, since the persistence length and the degree of polydispersity are the factors which must be determined by some independent methods. The smaller DNA chains are known to behave in a more rigid rodlike manner, as the ionic strength is lowered to, *e.g.*, 2×10^{-4} M as com-

pared with 0.2 M NaCl.[36,37] Thus the problem of the backbone chain flexibility may be resolved by using low ionic solutions, where, however, the light scattering and viscosity measurements become unreliable or impossible. In this case, the transient electric birefringence and dichroism techniques may be fully utilized.[36,37]

As is shown in Figure 1, a large quantity of 10 mg or more is necessary for a single light scattering or viscosity measurement, indicating that the preparation of monodispersed samples by the restriction-enzyme fragmentation method is prohibitive; hence, the use of sonicated sDNA samples, once well-fractionated, is recommendable. The $\bar{M}_w$ *vs.* $[\eta]$ relationship is firmly established in this work. The problem of polydispersity may be experimentally resolved by employing the refined gel electrophoretic techniques.[13]

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