Sonication, Fractionation, and Molecular Weight Distribution of Alternating Polydeoxyribonucleotides: Poly(dG-dC) · Poly(dG-dC) and Poly(dA-dT) · Poly(dA-dT)[†]

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ABSTRACT: To obtain well-defined rodlike, low-molecular-weight nucleic acid samples with the narrow distribution, the sonication of Poly(dG-dC) · Poly(dG-dC) and Poly(dA-dT) · Poly(dA-dT) was carried out at 105 W and at 0°C for totally 10 min. Each sample was fractionated into five to six fractions by Sephacryl S-500HR gel-filtration. The molecular weight distribution and weight-average molecular weight, M_w , of the unirradiated, irradiated, and fractionated samples were determined by the gel-permeation-chromatography/low-angle-laser-light-scattering method. Values of M_w were reduced from 5.7×10^5 to 1.6×10^5 for Poly(dG-dC) · Poly(dG-dC) and from 7.7×10^5 to 1.1×10^5 for Poly(dA-dT) · Poly(dA-dT) by sonication. The degrees of polydispersity, M_w/M_n , of sonicated but unfractionated samples were 1.3 and 1.8, respectively, differing from 2, the value expected for random scission. M_w/M_n of sonicated samples was further narrowed to a 1.04–1.42 range by the gel filtration. Compared with the measured molecular weight distributions, the Schulz–Zimm distribution function was adequate to describe sonicated but unfractionated samples, but the logarithmic-normal distribution represented the experimental distribution of fractionated samples. The double-helical structures of fractionated samples were found to be intact in all cases by various treatments.

KEY WORDS Poly(dG-dC) · Poly(dG-dC) / Poly(dA-dT) · Poly(dA-dT) /
Sonication / Fractionation / Gel-Permeation-Chromatography/Low-Angle-
Laser-Light-Scattering (GPC/LALLS) / Molecular Weight Distribution /

Purines are hydrogen-bonded to pyrimidines to form adenine-to-thymine (A·T) and guanine-to-cytosine (G·C) base pairs in DNA duplex, and the A·T pair melts easier than the G·C pair.¹ The difference in base composition should then be reflected in other properties such as flexibility, base stacking, and interaction with small molecules. For example, Poly(dA-dT) · Poly(dA-dT) duplex is reported to be more flexible than natural DNA,² whose persistence length is estimated at 45 to 55 nm in 0.2 M NaCl.^{3–5} Comparative studies of the solution conformation of deoxyribonucleotides with a simple base-sequence is needed for

clarifying the role of base composition in flexibility. For this purpose, well-defined samples that are closely monodisperse and remain double-stranded should be prepared.

Large amounts of double-helical, calf thymus DNA samples with the narrow distribution could be prepared by sonication and precipitation fractionation procedures of the high molecular weight sample.^{6,7} Well-defined, double- and triple-stranded polyribonucleotide samples were also obtained with the same method.^{8,9} These samples in the range of 3700 to 80 base pairs were examined for solution properties such as chain flexibility, rotational

[†] This is Part VII of Ultrasonic Scission of Deoxyribonucleic Acid in Aqueous Solution. For Part VI, see ref 9.

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diffusion constant, electric dipole moments by light scattering, circular dichroism, electric dichroism, and electric birefringence.¹⁰⁻¹⁴ The preparation of large amounts made possible to investigate the adsorption of DNA and polyribonucleotides onto microspheres such as a polystyrene latex.¹⁵ The preparation of monodisperse oligodeoxyribonucleotides has recently become easy by a DNA synthesizer, but the amount is too small to carry out many physicochemical measurements. It is exceedingly difficult to synthesize a monodisperse duplex longer than 200 base pairs. The chain-length dependence is important.^{10,12,13,16} To study solution conformation, enzymically synthesized high-molecular-weight polydeoxyribonucleotides (the range from 1×10^5 to 1×10^6) have been used, but these samples usually contain fragments of different sizes.

Sonication is suited for adjusting molecular weights of commercial samples in large quantities, as described in a previous paper.⁶ The sonication of double-stranded polydeoxyribonucleotides in small quantities remains to be attempted. The same method should be applied to precious samples in small amounts (*ca.* 10 mg). A preparative gel filtration (GF) method is now combined with the sonication of a sample, instead of precipitation fractionation which was previously described.⁷ Each method is commonly used in nearly every branch of biological science, but some basic problems still remain; for example, the effects of ultrasound on biopolymer and changes of the molecular weight distribution by sonication and fractionation.

In this study, two alternating polydeoxyribonucleotides, Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT), were sonicated and fractionated through a gel column with two objectives: (1) to establish a sample preparation method on a relatively small scale (*ca.* 10 mg) and (2) clarify the potential of sonication and fractionation on the basis of molecular weight distribution. A gel-permeation-chromatography/low-angle-laser-light-

scattering (GPC/LALLS) method¹⁷ is utilized for determining molecular weight distribution. The reduction of molecular weight by ultrasonic irradiation, and changes of polydispersity by fractionation were examined by the GPC/LALLS method. The distributions of the sonicated and fractionated samples are compared with theoretical logarithmic-normal and Schulz-Zimm distributions.¹⁸⁻²² The experimental results are discussed on the basis of molecular weight distribution.

EXPERIMENTAL

Materials and Preparation of Polydeoxyribonucleotide Solutions

Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) samples, each with 200 absorbance units, were purchased from Pharmacia LKB Biotechnology (Sweden) and Sigma Chemical Co. (U.S.A), respectively. Other chemicals are all of reagent grade.

A stock solution (10 mL) was carefully prepared at a concentration of *ca.* 1 mg mL⁻¹ in an ice bath. The concentration was spectrophotometrically determined with an absorption

Figure 1. Base-pairs of alternating Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b). G, C, A, and T stand for guanine, cytosine, adenine, and thymine, respectively. dR stands for deoxyribose.

coefficient of 7100 at 254 nm for Poly(dG-dC)·Poly(dG-dC) and 6600 at 262 nm for Poly(dA-dT)·Poly(dA-dT),^{23,24} whose base pairs are shown in Figure 1.

Sonication of a Small Amount of Sample Solution

The large-scale sonication procedure⁶ was modified to apply to small-volume polydeoxyribonucleotide solutions (10 ml). A sample solution was irradiated at 105 W and at 20 kHz with a micro chip (the diameter: 3 mm) of a Tommy Seiko model UR-200P sonicator in a polypropylene-made centrifuge tube (the volume: 15 ml) in place of a rosette vessel (the volume: 250 ml).⁶ 30-second irradiation and 5-min resting cycle were repeated twenty times (totally 10 min) under a helium gas atmosphere.

Molecular Weight Fractionation of Sonicated Samples

The sonicated samples were fractionated at 20°C by the GF method. Six bed volumes of a eluent (0.2 M NaCl) were introduced into a column packed with Sephacryl S-500HR gel (the height: 32.5 cm) for equilibration. Each 2-ml sonicated sample was loaded per run on the gel bed and eluted by the eluent at a flow rate of *ca.* 10 ml hr⁻¹. The eluted samples were collected in 1 ml portions. The same operation was repeated three times (totally 6 ml). Absorbance of the eluted samples was monitored at 290 nm on an UVIDEC-100II spectrophotometer (Japan Spectroscopic Co., Japan). The reproducibility of elution was confirmed by chromatograms. Based on the profiles, the eluted samples were separated into 6 fractions for Poly(dG-dC)·Poly(dG-dC) and 5 fractions for Poly(dA-dT)·Poly(dA-dT), being numbered from first (F1) to the last.

Purification of Fractionated Samples

Ion exchange chromatography was performed with a column packed with DEAE Bio-Gel A (the volume, *ca.* 10 ml) at room

temperature.²⁵ Each fractionated polydeoxyribonucleotide sample was applied onto the column to be adsorbed to the gel containing 0.2 M NaCl. Four bed volumes of 0.2 M NaCl were passed through the column to wash out unadsorbed contaminants. The adsorbed, sonicated sample was finally eluted with eluent (3 M NaCl) 3 ml each for 2 to 3 times.

Measurements

Absorption Spectra and Thermal Melting Profiles. The ultraviolet spectra of the sonicated samples were measured at 7°C on a Shimadzu Model UV-250 spectrophotometer controlled by an NEC PC9801VM2 microcomputer *via* a GPIB. Melting profiles of the samples were photometrically measured at 280, 260, and 235 nm. Solution temperature was raised at a rate of *ca.* 1°C/min⁻¹ by circulating temperature-controlled water around the cell.

GPC/LALLS. For unirradiated, irradiated, and fractionated samples, molecular weight distributions were determined at 30°C by the GPC/LALLS system, whose block diagram is shown in Figure 2. A sample solution (concentration: 0.29–0.08 mg ml⁻¹) was injected into a sample-loop of 0.5 ml, and loaded onto two separation-columns (Tosoh Co.

Figure 2. Block diagram of the Tosoh GPC/LALLS system. S, syringe; B, injection bulb; Solv, solvent; P, dual pump; SL, sample loop; CO, column oven; G, guard column; GPC, gel permeation chromatography column; Flt, membrane filter; LALLS, low angle laser light scattering photometer; RI, differential refractometer; MC, microcomputer with display.

TSK gel G-DNA-PW) connected in series, and eluted with a 0.2 M NaCl eluent at a flow rate of 0.6 mL min^{-1} . Changes in the refractive index and light scattering of the separated sample were monitored online by a Tosoh LS-8000 low-angle-laser-light-scattering photometer equipped with a differential refractometer.^{8,9} The molecular weights and concentrations of eluted components were evaluated to determine the molecular weight distribution. The instrumental constant was determined with bovine serum albumin (BSA) (Miles Inc. Diagnostics Div., U.S.A.) with the molecular weight of 66250, as the primary standard, and with polyethylene oxide (Tosoh Co.) with the weight-average molecular weight of 3.4×10^5 as the secondary standard.

RESULTS AND DISCUSSION

Effect of Ultrasonic Irradiation on Duplexes

Chemical Stability of Double-Helical Structure. Figure 3 shows the absorption spectra of Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) in 0.2 M NaCl at 7°C before and after sonication. The spectrum of unsonicated Poly(dG-dC)·Poly(dG-dC) (denoted as hGC) showed a maximum at 256 nm and a minimum at 227 nm (Figure 3a). After sonic irradiation at 105 W, the spectrum clearly coincided with that of the unsonicated sample. When the sonicated sample was denatured or chemically modified, the UV spectrum should be inevitably changed. However, this is not the case. The spectrum of the unsonicated Poly(dA-dT)·Poly(dA-dT) (denoted as hAT) sample showed a maximum at 262 nm and a minimum at 234 nm under the same conditions (Figure 3b), coinciding with the spectrum of the sonicated sample. The spectra of Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) clarified that neither the bases were chemically modified nor the duplex separated by sonication when the ultrasound was irradiated under the conditions used in this study (see EXPERIMENTAL).

Figure 3. Absorption spectra of double-stranded Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b) in 0.2 M NaCl at 7°C before and after ultrasonic irradiation at 105 W for a total of 10 min. Each spectrum is normalized to unity at maximum. Unsonicated and sonicated samples are indicated by solid lines and circles, respectively.

Thermal Stability of Double-Helical Structure. The melting of a duplex strongly depends on the concentration of added salt.¹ Therefore, the irradiation conditions should be carefully selected to avoid denaturation. In this study, the ultrasound was irradiated at high ionic strength of the sample solution (0.2 M NaCl) and at low temperature (*ca.* 0°C). Figure 4 shows melting profiles of Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) at 280, 260, and 235 nm in 0.2 M NaCl before and after irradiation. Absorbance of the hGC solution was hardly affected at any wavelength by increase in temperature from 7 to 90°C (Figure 4a), while a calf thymus DNA sample of comparative size melted at *ca.* 86°C at the same ionic strength.¹ The hGC sample began to melt from *ca.* 73°C, as salt concentration was decreased to 0.4×10^{-3} (not shown here). The melting profile of sonicated Poly(dG-dC)·Poly(dG-dC) (denoted as sGC) solution agreed with that of the h-sample, indicating no strand separation to be induced by sonication.

Figure 4. Thermal melting profiles of double-stranded Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b) in 0.2 M NaCl. Absorbances of sonicated samples are at 280 (○), 260 (△), and 235 (□) nm and solid lines indicate those of the corresponding unsonicated samples. Note that neither unsonicated nor sonicated Poly(dG-dC)·Poly(dG-dC) sample melts at any condition in this work.

The hAT sample began to melt at *ca.* 60°C and the melting was completed at 70°C (Figure 3b). The difference in melting curves between Poly(dG-dC) and Poly(dA-dT) duplexes would be due to the strength of hydrogen bonds (see Figure 1). The melting profile of the hAT sample was similar to that of the sonicated sample (denoted as sAT).

Molecular Weight Fractionation of Sonicated Samples by Gel Filtration

Chromatograms of Unsonicated and Sonicated Samples. Figure 5 shows Sephacryl S-500HR gel filtration chromatograms of Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) samples before and after sonication. The hGC sample was eluted at elution volume V_e near 37 ml, while the corresponding sGC sample was eluted at the V_e of 44 ml (see Figure 5a). Since the low molecular weight sample slowly eluted, the molecular weight of hGC sample was evidently reduced by ultrasonic irradiation. The half-width of a chromatogram is a qualitative index of the

Figure 5. Gel-filtration chromatograms of Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b). Unsonicated (—) and sonicated (-----) samples, gel-filtrated with Sephacryl S-500HR gel at 20°C. Absorbance was monitored at 290 nm. Dotted lines: void volume V_0 at 26 ml and void plus inner volume V_s at 62 ml determined with high-molecular-weight DNA and sodium benzenesulfonate.

degree of molecular weight distribution.⁶ Values of half-width were 5.3 ml for the hGC sample and 10.4 ml for the sGC sample, suggesting that the polydispersity of the unsonicated sample is lower than that of the sonicated one. In contrast, the half-width of the AT sample became narrower from 13.6 to 10.5 ml by the sonication (Figure 5b). The degree of polydispersity was lowered by sonication, quantitative difference between unsonicated and sonicated samples being clarified by GPC/LALLS measurement later. Two peaks in the chromatogram of sAT sample were observed: one 45 ml and the other 63 ml. Judging from the hyperchromicity at 260 nm of the sAT sample (51%) close to that of the hAT sample (47%), the slowly eluted component of sAT sample at the V_e of 63 ml appeared intact.

Molecular Weight Fractionation of Sonicated Samples. The void volume V_0 and void plus inner volume V_s of gel bed were determined to be 26.2 and 61.9 ml, respectively, by calf thymus DNA as a high-molecular-weight marker and sodium benzenesulfonate as a low-molecular-weight marker (Figure 5). K_d , defined as $K_d = (V_e - V_0)/(V_s - V_0)$, of each s-sample was

about 0.5, indicating the present gel to be appropriate for separating sonicated samples. Each eluted s-sample was fractionated into five to six fractions on the basis of the elution profile of the chromatogram.

Molecular Weight Distributions Determined by GPC/LALLS

Effects of Sonication on Molecular Weight Distribution. Figure 6 shows the molecular weight distributions of irradiated Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) samples together with the original distributions as reference. The hGC sample shows the degree of polydispersity of 1.16, expressed as the ratio of weight- to number-average molecular weight, M_w/M_n , suggesting that the original sample was well fractionated. The commercial hAT sample shows a wide distribution with M_w/M_n of 2.01, probably because it was an unfractionated sample. Our sonication study of DNA clarified a lower limit of molecular weight.^{6,7} The irradiation reduced the weight-average molecular weight M_w from 5.7×10^5 to 1.6×10^5 . M_w of AT sample was also reduced from 7.7×10^5 to 1.2×10^5 . These data are summarized in Table I with the degree of polydispersity, M_w/M_n and M_z/M_w (the z- to

weight-average molecular weights).

The effects of sonication on the degree of polydispersity was more complex. Prior to fractionation, M_w/M_n was increased from 1.16 to 1.29 for the GC sample by sonication but decreased from 2.01 to 1.79 for the AT sample. Then, M_w/M_n appeared to converge to a constant value of 1.4—1.3 for both samples, as sonication proceeded (Table I). M_w/M_n obviously differs from 2.0, expected for the randomly scissored process,²¹ indicating non-random scission of the double-helical chain. Numerical calculation by Ballauff and Wolf²⁶ reveal that M_w/M_n converges to about 1.4 after a sufficiently long period of irradiation regardless of the initial M_w/M_n , if a linear chain is scissored only at the midpoint. Such non-random scission may occur in the present case (detailed mechanism will be reported later). The small-scale sonication method is suited for reducing molecular weight and, particularly, narrowing the degree of polydispersity.

Effects of Fractionation by Gel Filtration on Molecular Weight Distribution. Figure 7 shows the molecular weight distributions of fractionated samples with those of unfractionated samples. M_w as well as M_w/M_n values of fractionated sGC samples became smaller, as separation proceeded. The present GF method is proved suitable for fractionation of a small quantity. It should be emphasized that the degree of polydispersity is fairly narrow for all gel-filtrated samples (e.g., 1.04 for F1 and 1.11 for F5), compared to the data of DNA samples obtained by precipitational fractionation.^{7,8} The GF method is also effective for narrowing the degree of polydispersity for an sAT sample. Thus, gel-filtrated fractionation is recommendable for small amounts of samples, because they are separated according to size, but not polynucleotide species.

The precipitational fractionation method has advantages that a large amount of sample can be treated in a batchwise operation and the special apparatus is unnecessary for

Figure 6. GPC/LALLS-determined molecular weight distributions of Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b) before and after ultrasonic irradiation at 105 W. The weight fraction, g_w , was plotted against the common logarithm of molecular weight, $\log(M)$. Each area was normalized to unity for unsonicated (—) and sonicated (----) samples.

Table I. Weight average molecular weight, M_w and polydispersities, M_w/M_n and M_z/M_w , of unirradiated, irradiated but unfractionated, and fractionated polydeoxyribonucleotides

Samples	Fractions	$M_w/10^4$	bp ^a	M_w/M_n	M_z/M_w	Y^b/mg
Poly(dG-dC)·Poly(dG-dC)	h ^c	57.3 ± 2.3^d	868	1.16 ± 0.03^d	1.17 ± 0.04^d	—
	s ^c	16.2 ± 0.6	245	1.29 ± 0.04	1.24 ± 0.04	—
	F1	30.9 ± 1.2	468	1.04 ± 0.03	1.04 ± 0.03	0.36
	F2	22.7 ± 0.9	344	1.06 ± 0.03	1.06 ± 0.03	1.09
	F3	18.0 ± 0.7	273	1.07 ± 0.03	1.06 ± 0.03	1.15
	F4	14.2 ± 0.6	215	1.11 ± 0.03	1.11 ± 0.03	1.24
	F5	11.9 ± 0.5	180	1.10 ± 0.03	1.11 ± 0.03	1.41
	F6	8.54 ± 0.34	129	1.42 ± 0.04	1.60 ± 0.05	0.73
						(5.98)
Poly(dA-dT)·Poly(dA-dT)	h	77.0 ± 3.1	1170	2.01 ± 0.06	1.55 ± 0.05	—
	s	11.1 ± 0.4	168	1.79 ± 0.05	1.53 ± 0.05	—
	F1	21.5 ± 0.9	326	1.06 ± 0.03	1.05 ± 0.03	0.40
	F2	15.1 ± 0.6	229	1.09 ± 0.03	1.08 ± 0.03	1.03
	F3	11.5 ± 0.5	174	1.12 ± 0.03	1.16 ± 0.03	0.94
	F4	8.70 ± 0.35	132	1.14 ± 0.03	1.12 ± 0.03	1.21
	F5	6.89 ± 0.28	104	1.34 ± 0.04	1.61 ± 0.05	0.63
						(4.21)

^a Number of base pairs was evaluated by $M_w/660$.^b A recovery was defined as the photometrically-determined weight of a fractionated sample. Values in parentheses are the sum of recoveries of all fractionated samples.^c h and s denote unirradiated and irradiated but unfractionated samples, respectively.^d Error results from evaluation of the area of chromatogram. **Figure 7.** GPC/LALLS-determined molecular weight distributions of Poly(dG-dC)·Poly(dG-dC) (a) and Poly(dA-dT)·Poly(dA-dT) (b) before and after fractionation. Sonicated but unfractionated (—) and fractionated (—) samples. Numerals denote fraction numbers of gel-filtrated samples. Other notations are the same as in Figure 6.

fractionation.⁷ The method has disadvantages; a narrowly polydisperse sample can be obtained only by decreasing the concentration of the mother solution and precipitating successively the smallest possible amounts.²⁷ The narrowing of molecular weight distribution is less effective for the higher molecular weight samples. The choice of precipitant possibly influences the degree of polydispersity of the fractionated sample. The precipitant should be selected so that it induces no denaturation of biopolymers. Although each sonicated sample was fractionated only into 5–6 fractions in this study, the GF method provided the excellent result that M_w/M_n was narrow (1.04–1.14 in Table I). Conditions, such as species of gel, added volume, concentration of sample, and flow rate, can be easily adjusted for separation by the GF method. A large amount of closely monodisperse sample may be easily prepared, if a large preparative column is used.

Figure 8. Relationship between M_z/M_w and M_w/M_n for unsonicated, sonicated but unfractionated, fractionated polydeoxyribonucleotides. Measured points: circles for Poly(dG-dC)·Poly(dG-dC) and triangles for Poly(dA-dT)·Poly(dA-dT). Closed, half-closed, and open symbols denote unsonicated, sonicated but unfractionated, and fractionated samples, respectively. Theoretical curves: logarithmic-normal type, (---) and Schulz-Zimm type, (—).

Polydispersity of Unirradiated, Irradiated, and Fractionated Samples. Figure 8 shows the relationship between M_z/M_w and M_w/M_n for unirradiated, irradiated, and fractionated samples. If a molecular weight distribution obeys a logarithmic-normal profile, M_z/M_w should be equal to M_w/M_n , as indicated by the broken line in Figure 8.¹⁸ If the experimental distribution obeys the Schulz-Zimm function, the relation between M_z/M_w and M_w/M_n is given as follows: $M_z/M_w = 2 - (M_w/M_n)^{-1}$ (solid line in Figure 8).^{19,20} M_z/M_w should be less than, or equal to, M_w/M_n for the Schulz-Zimm distribution. Compared with Schulz-Zimm and logarithmic-normal theoretical curves, the experimental values could be classified as a well-fractionated group with M_w/M_n less than 1.2, M_w/M_n larger than 1.2 and the M_z/M_w , and a third group. For the first group, M_w/M_n agrees with M_z/M_w within an error of 3%, indicating that the distribution type is difficult to distinguish from measured M_w/M_n and M_z/M_w . The second group consists of sonicated GC and AT samples, and a commercial AT sample, located in the vicinity of the theoretical Schulz-Zimm curve. In the last group, M_z/M_w is larger than M_w/M_n , because the molecular weight distribution of the last fractions (F6 for GC and F5 for AT) tends to

Figure 9. Comparison of experimental molecular-weight-distributions of sonicated but unfractionated polydeoxyribonucleotides with fitted theoretical functions. (a): Poly(dG-dC)·Poly(dG-dC) sample ($M_w = 1.62 \times 10^5$, $M_w/M_n = 1.29$). (b): Poly(dA-dT)·Poly(dA-dT) sample ($M_w = 1.11 \times 10^5$, $M_w/M_n = 1.79$). Two theoretical logarithmic-normal (---) and Schulz-Zimm (.....) functions were calculated from M_w and M_w/M_n obtained from experimental distributions (—).

tail toward higher molecular weight. The distribution type can be roughly estimated from the relationship between M_z/M_w and M_w/M_n .

Molecular Weight Distribution of Sonicated and Fractionated Samples and Analytical Distribution Functions

Features of Molecular Weight Distribution of Sonicated Samples. Figure 9 shows the GPC/LALLS-determined molecular weight distribution of sonicated GC and AT samples together with analytical logarithmic-normal and Schulz-Zimm functions. These analytical distributions were chosen, because (1) each has only two adjustable parameters, (2) each is easy to treat, or calculate, mathematically, and (3) both functions describe a very narrow or very wide distribution. The measured distribution of sGC sample shows a maximum, closely located at $M_w (= 10^{5.17})$, and tails toward lower molecular weight (Figure 9a). However, the corresponding logarithmic-normal dis-

Figure 10. Comparison of experimental molecular-weight-distributions of fractionated polydeoxyribonucleotides with the corresponding theoretical functions. Fractionated Poly(dG-dC)·Poly(dG-dC) (F3, $M_w = 1.80 \times 10^5$, $M_w/M_n = 1.07$) (a) and (F6, $M_w = 8.54 \times 10^4$, $M_w/M_n = 1.42$) (b). Fractionated Poly(dA-dT)·Poly(dA-dT) (F3, $M_w/M_n = 1.15 \times 10^5$, $M_w/M_n = 1.12$) (c) and (F5, $M_w = 6.89 \times 10^4$, $M_w/M_n = 1.34$) (d). Other notations are the same as in Figure 9.

tribution yields the maximum at M lower than the M_w . This is an important feature of the theoretical distribution. The corresponding Schulz–Zimm distribution could reproduce the experimental profile well.

The measured distribution of sonicated AT sample is not symmetric but skewed toward the lower molecular weight side (Figure 9b). This distribution can be adequately approximated by Schulz–Zimm but not by logarithmic function. Thus, the Schulz–Zimm function may be concluded adequate to express the molecular weight distributions of the present sonicated samples. The features observed for both samples may be associated with a common mechanism of ultrasonic scission.

Molecular Weight Distributions of Fraction-

ated Samples. Figure 10 shows the molecular weight distributions of two fractionated sGC samples, F3 (a) and F6 (b), and fractionated sAT samples, F3 (c) and F6 (d), together with the corresponding logarithmic-normal and Schulz–Zimm functions. The measured profile of GC sample (F3) is sharp but widely-trailed and slightly different from the both fitted distribution curves. However, M_z expected from the fitted distribution is in agreement with the measured one within experimental error, because the dispersity is low. Thus, the molecular weight distribution of well-fractionated samples can be adequately expressed either by logarithmic-normal or by Schulz–Zimm distribution. The same trend was also observed for the well-fractionated AT sample (F3).

The measured distribution curve of the last GC fraction (F6) shows a maximum on the lower side of the peak of the fitted functions. Thus, M_z/M_w is estimated to be 1.60, larger than 1.42 for the corresponding M_w/M_n value. If theoretical distributions are fitted to measured profiles to calculate the degree of polydispersity, M_z/M_w may be underestimated as 1.42 for the log-normal type and 1.30 for the Schulz–Zimm type. The logarithmic-normal function may be recommended as a better approximation for molecular weight distributions of the most slowly eluted fractions.

CONCLUSION

A small-scale preparation technique of the low-molecular-weight polydeoxyribonucleotides (1200–100 base pairs) with narrow molecular weight distribution was established. Samples were scissored at *ca.* 10 mg/10 ml by ultrasonic irradiation without chemical damage and denaturation under the conditions used in this study. The sonicated samples could be fractionated by gel filtration with an Sephacryl S-500HR gel. The molecular weight and distribution of each sample could be char-

acterized for a small quantity by the GPC/LALLS method. The measured molecular weight and distribution of sGC and sAT samples can be fitted by the Schulz-Zimm function, while the distribution of fractionated samples is closer to the logarithmic-normal type. For fractionated samples, M_w were in the range of $(7-31) \times 10^4$ and M_w/M_n were lower than 1.14. These nearly monodisperse samples will be utilized for studying solution properties.

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