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POLYDISPERSITY AND CHAIN BREAK EFFECTS ON DNA INTRAMOLECULAR RENATURATION

Francisco MINGOT, M. Isabel ACUÑA, Fernando GARCES and Carlos A. DAVILA

Group of Biophysics, Division of Fundamental Research, Junta de Energía Nuclear, Ciudad Universitaria, Madrid 3, Spain

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The influences of sample polydispersity and of internal chain breaks are introduced in the formal description of intramolecular renaturation phenomenology, enhancing the usefulness of the involved methodology. In our method, renaturation is induced not by cooling but by increasing the sodium concentration. The single-stranded size distributions for the two types of samples employed (haplotomic and diplotomically degraded) are discussed. In the case of haplotomic cleavage, the variable is the strand size in the 'segment' between consecutive nicks in either of the native DNA strands. Our equations have been obtained by considering that the arrangement of DNA sequences may be approximately taken as random. These equations provide a good description of experimental data and a reasonable value (about 1000 base-pairs) for the size of the thermalite sequence, but show low sensitivity to departures from the random arrangement of sequences.

1. Introduction

The denaturation of DNA occurs in a cooperative way, not for whole molecules but for sequences of a given average length (sometimes called thermalites or cooperative units), giving rise to the well known partial denaturation and intramolecular renaturation phenomena [1–12].

The interesting study of Vizard [12] on the relationship between the denatured mass fraction (which we will call D) and the fraction of molecules that strand-separate (here called I) did not take into account the size heterogeneity inherent to any purified bacterial or mammalian DNA sample and ignored the influence of chain breaks.

In this report, we try to show that an approximate formal picture can be developed that overcomes these limitations, thus enhancing the capabilities of the methodology.

2. Theory

Our formal description of the intramolecular denaturation reversibility relies on the results of Vizard [12] about the arrangement of sequences in the bacterial genome. His work on the relationship between I and D shows that the most stable thermalites may be considered as being randomly distributed along the bacterial genome of *Escherichia coli*. Moreover, his study of the linear renaturation suggests that for bacterial DNA all sequences of any stability are arranged in a way not easily distinguishable from an uncorrelated mode.

Therefore, for bacterial DNA, a random sequence stability distribution may be considered, as already proposed by Elton [13]. Nevertheless, such an uncorrelated distribution must only be viewed as an approximative formal picture of the actual distribution.

Let A be the average molecular weight of a thermalite strand, N the number of thermalites in the sample and $N(T)$ the number of them that denature at a given temperature T. Obviously, the denatured mass fraction will be $D = N(T)/N$.

Let us assume momentarily a monodisperse population of molecules without internal nicks. As irreversibility appears when the two DNA strands separate completely, the irreversibly denatured

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fraction will be the fraction of molecules having all its thermalite sequences denatured. Therefore, if the molecules have M/A thermalites (M being the molecular weight of a strand) and a random sequence arrangement is considered, then the irreversibly denatured or strand-separate fraction will be $I = D^{(M/A)}$.

This is only true for molecules of size greater than A, since if the thermalite behaves like a cooperative unit, then a molecule of size A or less must be completely native or denatured at any temperature and the irreversibly denatured fraction of these small molecules must be the same as the whole denatured fraction D.

Therefore, for a polydisperse sample, the following general expression may be written

$$I = D\int_0^A \mathrm{d}F(M_i) + \int_A^\infty D^{(M_i/A)}\,\mathrm{d}F(M_i) \qquad (1)$$

where $F(M_i)$ is the mass fraction size distribution function of single-stranded chains ($\mathrm{d}F(M_i)$ being the corresponding density function) as I and D are mass fractions obtained by observing an optical property.

However, $F(M_i)$ is a meaningless function if there is no simultaneous knowledge of the frequency and arrangement of intrachain breaks, since breaks have an important quantitative effect. As may be realized, samples of equal native size display very different intramolecular renaturation behavior depending on their single-break densities [9]. The distribution function $F(M_i)$ exhibits a defined relationship with the frequency and arrangement of intrachain breaks for only two particular types of samples.

Sufficiently haplotomically cleaved DNA solutions belong to one of these types of samples. It is well established that for these samples, internal chain breaks are randomly distributed and that the mass fraction size distribution of single-stranded chains is [14,15].

$$F(M_i) = 1 - (1 + (M_i/M_n))\exp(-M_i/M_n) \qquad (2)$$

$F(M_i)$ being the mass fraction of strands of size equal to or less than M_i, and M_n the corresponding number average molecular weight.

Nevertheless, the relevant variable in the intramolecular renaturation phenomenon is not

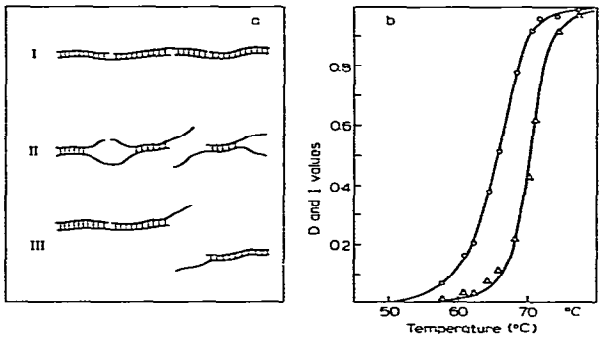

Fig. 1. (a) Reversibility of DNA denaturation. (I) State at low [Na^+] and low temperature. (II) State at low [Na^+] and a temperature near the melting point. (III) State at the same temperature as in II but after increasing [Na^+] which induced an increase in melting temperature of about 20°C. The sample recovers the native structure with the exception of the segment which underwent complete separation. (b) Partially reversible (○) (D) and irreversible (△) (I) denatured mass fractions for a sonicated DNA of *E. coli* of $M_{50} = 2.6 \times 10^6$. The curve through triangles has been calculated using eq. 7, with $A = 1040$.

the average size between interruptions in the same strand but the size of one strand in the segment between two consecutive breaks in either of the two strands of the native DNA molecule (M_{sn}). The following consideration supports this assertion. If all thermalites in the segment become denatured, then the two strands of this segment move away and the segment cannot become renatured even if some renaturation occurs at other zones in the molecule (see fig. 1a) [8,9,16]. Renaturation may then take place through a nucleation process, but this is kinetically impossible under our experimental conditions [9,17] (see section 3).

As in haplotomically degraded samples intrachain breaks are randomly arranged, M_{sn} must be half the M_n value. Moreover, in this type of sample, $M_n = 0.6M_{50}$ [15] and hence $M_{sn} = 0.3M_{50}$, where M_{50} is the molecular weight derived from the 50% point in the sedimentation coefficient distribution. As a consequence, after haplotomic cleavage of a DNA sample, the mass fraction size distribution of the segments will be

$$F(M_i) = 1 - (1 + (M_i/0.3M_{50}))\exp(-M_i/0.3M_{50}) \qquad (3)$$

therefore

$$I = D(1-F(A)) + \int_A^\infty D^{(M_i/A)} \frac{M_i}{(0.3M_{50})^2} \exp\left[\frac{-M_i}{(0.3M_{50})}\right] dM_i \quad (4)$$

and by integrating

$$I = D\left\{1-\left[\frac{A}{0.3M_{50}} + 1 - \frac{A/(0.3M_{50})}{1-(0.3M_{50}\ln D)/A} - \frac{1}{(1-(0.3M_{50}\ln D)/A)^2}\right]\exp\left[\frac{-A}{0.3M_{50}}\right]\right\} \quad (5)$$

The second type of sample with intrachain breaks under control is that which was originally highly polymerized and then sufficiently degraded by a diplotomic degradation agent. In these samples the intrachain break density may be neglected. Using a numerical χ^2-minimization procedure, we have determined the size distribution for our sonically degraded samples as being close to [18]

$$F(M_i) = 1-\exp\left(-0.64(M_i/M_{50})^2 - 0.07\right) \quad (6)$$

consequently, the irreversibly denatured fraction for this type of sample will be

$$I = D(1-F(A)) + \int_A^\infty D^{(M_i/A)} \frac{1.28M_i}{M_{50}^2} \times \exp\left(-0.64(M_i/M_{50})^2 - 0.07\right) dM_i \quad (7)$$

The integral in eq. 7 cannot be analytically performed and has been computed numerically.

Davis and Phillips [19] reported the production by sonication of samples whose native size was describable by the most probable Schulz distribution, but this was achieved after much more extensive degradation than we had induced. Anyway, our formulation accepts any distribution characteristic of a set of samples with the sole condition of the absence of intrachain nicks.

3. Experimental section

3.1. Materials

Calf thymus DNA was obtained following the procedure of Kay et al. [20] and *E. coli* DNA by a procedure described elsewhere [21]. All other chemicals were reagent grade. Diplotomic degradations were accomplished by ultrasonic treatment [18] under conditions similar to those used by Davis and Phillips [19] but treatment times were much shorter (< 3 min). Haplotomic treatments were performed by DNase digestion or γ-irradiation [15].

3.2. Molecular weights

All details about the determination of single-stranded molecular weights have been reported elsewhere [21]. Sedimentation coefficient distributions were obtained from boundary analysis [22]. To eliminate diffusion effects, the extrapolation $1/t \to 0$ [23] was performed. The extrapolation to infinite dilution was made when necessary.

3.3. Denaturation-renaturation

DNA samples in a solvent of a low sodium concentration (0.002 M Na^-) were heated in the sample compartment of a spectrophotometer up to a preselected temperature-induced absorbance increase. Then 1/100 vol. of a 4 M Na^+ solution was added, inducing instantaneous (≈ 3 s) intramolecular renaturation. All details of this procedure were previously reported [9]. The denatured fractions D and I were calculated from the hyperchromic effects before and after inducing renaturation.

4. Results and discussion

The description of single-stranded molecular weight distributions by the proposed functions is good for the two types of samples employed, as shown elsewhere [14,15] and as may be seen in fig. 2. The empirical distribution function used for diplotomically degraded samples lacks physical meaning when M_i approaches 0, but it does not matter, since we derive no conclusions from the parameters of this function. As shown in fig. 2a, our undegraded samples must be considered as haplotomically treated (even if their single-break frequencies are very low: 10^{-5}–10^{-4} single breaks per nucleotide) perhaps as a result of DNase action during the isolation procedure.

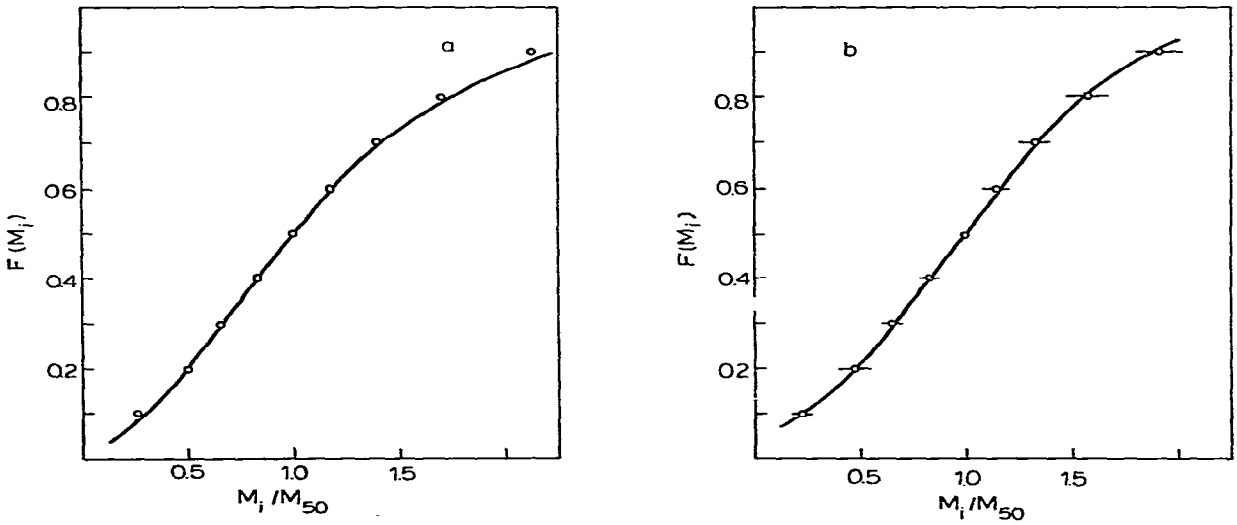

Fig. 2. Single-stranded DNA size distribution. (a) *E. coli* DNA not treated ($M_{50} = 16.5 \times 10^6$). Points are experimental data. The curve has been obtained from eq. 2. $F(M_i)$ is the mass fraction of molecules of size equal to or less than M_i (b) *E. coli* DNA sonicated down to various molecular weights. Points are averaged experimental values from six samples of different M_{50} values. The curve has been obtained from eq. 6. $F(M_i)$ as before.

We have studied the renaturation of haplotomic and diplotomically treated *E. coli* DNA samples. A typical experiment is depicted in fig. 1b. We have measured about six D, I pairs of values for every sample (total number of data points 50). The experimental I values were fitted to the theoretical ones obtained through eq. 5 or 7 (depending on sample characteristiques) by a numerical χ^2-minimization procedure. D and M_{50} were experimentally known and the size of the thermalite, A, was taken as an adjustable parameter. The mean experimental I error was estimated to be 0.1.

The χ^2 value was 36, indicating that the proposal of uncorrelated thermalite arrangement is admissible at the precision level of our results. The value found for the mean thermalite size was 940 $\pm$ 100 base-pairs.

Then we included the data for calf thymus DNA (41 data points more, total 91), obtaining a χ^2 value of 71 and a thermalite size of 1040 $\pm$ 130 base-pairs. Values of A within standard deviation were also obtained when the data for haplotomic and diplotomically degraded samples were fitted separately. The calculated values of I have been plotted vs. the experimental values in fig. 3.

The results obtained after including the calf thymus DNA data indicate that the relationship

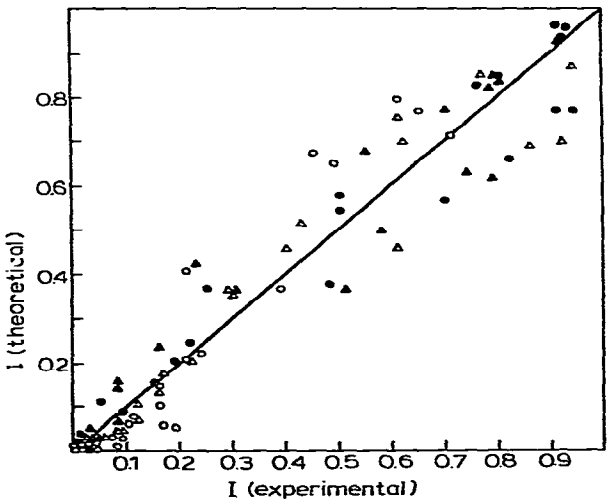

Fig. 3. Theoretical values of the irreversibly denatured mass fraction vs. the experimental values. (○, ●) Samples haplotomically degraded, (△, ▲) diplotomically cleaved. (○, △) *E. coli* DNA samples, (●, ▲) calf thymus DNA samples.

between I and D is rather insensitive to deviations from a random arrangement of sequences, since for calf thymus DNA the expected departure from randomness is greater than that for *E. coli* DNA. Nevertheless, we think that this insensitivity does not invalidate the practical purpose pursued here.

In the work of Vizard [12], only the most stable thermalites were considered as being randomly distributed, thus obtaining (using our symbols) $I = \exp((D-1)(M/A))$, which is asymptotic with our expression $I = D^{(M/A)} = \exp(\ln D(M/A))$ when D approaches 1. As may be realized, $D-1$ are the two first addenda in the Taylor series development of $\ln D$. Our formulas allow more rapid work, since for a given DNA sample, if the equations are valid over the entire range of D values, many determinations may be made for every sample. Nevertheless, our equations can be restricted to the melting zone where D is close to 1 (that zone where only the most stable thermalites remain native) when it would be suggested by knowledge of thermalite distribution.

Our expressions permit calculation of the I value corresponding to any D value if the DNA molecular weight and size distribution are known. Thus, information may be obtained about the maximum denaturation degree that a sample may attain compatible with an almost complete recovery of the native structure. Conversely, the single-stranded molecular weight may be easily obtained from D and I measurements.

The A value found is about half that estimated by Elton [13] but close to the results of Vizard [11,12], compatible with selective denaturation of genetic markers [24], electron microscopy denaturation patterns [11] and stability analysis results [25,26]. Rusell and Holleman [27] obtained a greater value from renaturation data, but they used equations with evident shortcomings. The A value also shows a suggestive coincidence with the DNA size at which melting from the ends may begin to be considered as the sole denaturation pathway [4].

One must emphasize the role of the segment which appears as being the relevant entity in size-dependent denaturation phenomenologies. We also want to note that intramolecular renaturation is the basis of the cheaper methodology that can be applied to the determination of very low levels of 'in vivo' DNA damage [28,33]. This is an encouraging situation.

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