Biophysical Chemistry 13 (1981) 275–281
North-Holland Publishing Company

A THERMODYNAMIC VIEW OF POLYMER DEGRADATION, AND ITS APPLICATION TO EXTENSIVE SONICATION OF DNA

A.B. HALL, J.R. CHRISTIE
Department of Physical Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia

and

D.R. PHILLIPS
Department of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia

Received 6 October 1980
Revised manuscript received 2 February 1981

A simple thermodynamic theory is developed, which predicts (in agreement with a wide variety of other theoretical approaches and experimental results) that for simple polymers the most probable Schulz distribution of fragments will be obtained in a polymer degradation process that is allowed to proceed to a dynamic equilibrium. When the same method is applied to a double-stranded polymer like DNA, however, it predicts that some narrowing of this distribution may occur in conjunction with a limited amount of base unpairing at the fragment termini. The compatibility of this prediction with the experimental results of long-time sonication of DNA is considered.

1. Introduction

The physical principles of ultrasound, its concomitant effect of cavitation and the mechanical effects of cavitation on biopolymers has been the subject of a recent extensive review [1]. Although DNA may be degraded by many different processes [1] when subjected to ultrasonic irradiation (e.g. local temperatures up to 10^4 K, local pressures up to 10 atm, photoelectric effects, formation of free radicals, sonoluminescence and chemiluminescence), Hughes and Nyborg [2] have convincingly demonstrated that the mechanical effect of streaming is the major cause of the initial degradation of polymers. Streaming at the probe tip can produce eddying motions of up to 10 m/s, and this results in hydrodynamic shearing of polymers [2].

Many authors have concluded that the degradation appears to be by double-strand scission [3–6], yielding macro-radicals [7], with little evidence for the formation of any denatured or single-stranded DNA accompanying the depolymerization [6,8]. Richards and Boyer [9] have shown that 90% of DNA degradation occurs at C–O bonds of the DNA backbone and 10% at P–O sites (no C–C breaks were detected). However, Shugalii and Fonarev [10] have clear experimental evidence which suggests that the degradation proceeds by random single-strand breaks which ultimately match up after sufficient sonication time, to yield degraded but native DNA with no single-strand breaks. The detailed mechanism by which DNA is degraded by ultrasonic irradiation therefore remains obscure.

The kinetics of the ultrasonic degradation of polymers has also been studied in an endeavour to gain insight into the nature of the degradation process, and this area has been thoroughly reviewed by Peacocke and Pritchard [11]. Several theories have been proposed which assume a first-order rate constant for degradation that is proportional to the chain length in excess of a limiting chain length. Linear experimental plots of this type have been obtained for the ultra-

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sonication of gelatin, polystyrene and DNA [11]. Analysis of the distribution curve of the sedimentation coefficient of DNA has indicated that DNA is degraded by approximately successive halving [12]. Peacocke and Pritchard [11] have therefore concluded that the initial degradation of DNA occurs by preferential rupturing of the double-strand near the middle of the molecule until the molecular weight decreases to a limiting molecular weight which is dependent on experimental conditions.

It is important to note that these theories are applicable only to the initial degradation of polymers, where mechanical, hydrodynamic shearing is the dominant process [1,6]. At longer sonication times, these linear relationships are no longer valid. This suggests that if sonication is continued past the predominantly hydrodynamic shearing stage, other degradation (and synthetic) processes could become important. This therefore means that none of the theories based on hydrodynamic shearing of polymers can be extended or extrapolated to predict the molecular weight or molecular weight distribution of a polymer solution after infinite ultrasonic exposure. However, it has repeatedly been observed that macromolecules do degrade to an apparently limiting molecular weight [6,11,13,14] after which time further ultrasonic radiation has little or no effect. Davis and Phillips [14] have shown that for DNA, a limiting molecular weight distribution is also obtained as well as an apparently limiting molecular weight. This limiting molecular weight distribution [14] appears to be approximated by the most probable Schulz distribution [15].

Many polymers, under a wide variety of conditions of degradation or growth, form a molecular weight distribution which corresponds closely to the most probable Schulz distribution [16,17]. It is therefore clear that this resulting distribution reflects some fundamental property of the system rather than a result of the particular nature of the particular growth/degradation process. Indeed, the Schulz distribution has been shown [17] to arise naturally from a very simple statistical model of a generalized growth/degradation process in which the simplifying assumption is that any monomer–monomer linkage is equally likely to be absent (or present).

The fact that a limiting molecular weight of DNA (and other polymers) is obtained after extensive sonication, suggests some type of equilibrium process. The insensitivity of the distribution obtained to the type of degradation process seems to indicate an essentially thermodynamic control of the resulting distribution.

In this paper we show an alternative thermodynamically based model, appropriate to many degradation processes, which also gives this same distribution as a consequence. More importantly, it can be readily extended to more complicated polymers. We particularly examine the case of a double-strand polymer, where it predicts a characteristically different distribution.

2. The model and its predictions for simple polymers

In formulating a thermodynamically based model to consider the equilibrium achieved after prolonged sonication of a polymer solution, we focus attention on the shock heating aspect of the sonication process. The view is taken that energy is added to and removed from the polymer molecule in a series of sharp bursts, in a process that is local on the bulk medium scale, but non-local on the molecular scale (at least to the point of being chemically non-specific).

The degradation will then be regarded as arising from a situation where, with the passage of a shock front, a polymer molecule is heated to a high temperature where it can react to form two smaller fragments – or to join with another nearby molecule [18]. These processes proceed for a short time until the shock front passes, the temperature drops rapidly, and the reactions cease until the passage of the next shock front. The result of sonication over a prolonged period will then be the production of a molecular weight distribution corresponding to the equilibrium distribution at some elevated temperature associated with the shock front – a local temperature that could never be achieved in the bulk medium.

Consider a simple linear polymer. The standard free energy of an n-mer in solution is given at a particular temperature T, by:

$$G_n^0 = a(T) + b(T)n. \tag{1}$$

The quantity a becomes increasingly negative with increasing temperature (i.e. higher temperatures favour smaller species). Suppose that the concentration (the number or molar concentration) of n-mers is C_n. Then the chemical potential for an n-mer is

$$\mu_n = a(T) + b(T)n + RT \ln C_n. \quad (2)$$

We can divide this quantity (essentially an energy) among the monomer units to give

$$\mu_n^{\mathrm{M}} = a(T)/n + b(T) + (RT/n) \ln C_n, \quad (3)$$

where μ_n^{M} is the chemical potential associated with each monomer unit in an n-mer. Now if the system is at equilibrium, the chemical potential associated with a monomer unit should be the same regardless of the size of n-mer it is incorporated in. Thus

$$a(T)/n + b(T) + (RT/n) \ln C_n = \text{const}\ (T). \quad (4)$$

The constant will depend on T and the total concentration of material present, but be independent of n. We absorb $b(T)$ and const(T) into $d(T)$ = const(T) $- b(T)$. Then

$$C_n = \mathrm{e}^{[nd(T) - a(T)]/RT}. \quad (5)$$

The total number of monomer units present per unit volume (C mole) is a known quantity, which remains constant throughout any degradation experiment. The quantity $d(T)$ on the other hand is completely unknown. It can be obtained from

$$C = \sum_{n=1}^{\infty} nC_n = \mathrm{e}^{-a(T)/RT} \sum n\mathrm{e}^{nd(T)/RT}. \quad (6)$$

Evaluating the sum yields

$$C = \mathrm{e}^{[d(T) - a(T)]/RT}(1 - \mathrm{e}^{d(T)/RT})^{-2}. \quad (7)$$

The quantity d must be negative in order for the sum to converge. Rearrangement gives:

$$\mathrm{e}^{d(T)/RT} = [2C + \mathrm{e}^{-a(T)/RT} - (\mathrm{e}^{-2a(T)/RT} + 4C\mathrm{e}^{-a(T)/RT})^{1/2}]/2C = \beta(C, T) \quad (8)$$

and we finally obtain

$$C_n = \mathrm{e}^{-a(T)/RT}\beta^n(C, T), \quad (9)$$

which defines the molecular weight distribution. The degree of polymerization, DP, is introduced, and moments of the distribution calculated as follows:

$$\bar{M}_j = \sum_{n=1}^{\infty} m_n^j C_n \Big/ \sum_{n=1}^{\infty} m_n^{j-1} C_n = M^{\mathrm{M}} \sum_{n=0}^{\infty} n^j \beta^n \Big/ \sum_{n=0}^{\infty} n^{j-1}\beta^n - 0^{j-1}, \quad (10)$$

where M^{M} is the molecular weight of a monomer unit.

The summation limit is changed from 1 to 0 to make use of the identity

$$\sum_{n=0}^{\infty} n^j y^n = (y\mathrm{d}/\mathrm{d}y)^j (1 - y)^{-1},$$

where $|y| < 1$. The term 0^{j-1} becomes important in calculating $\bar{M}_1$.

The first three moments give the number, weight and Z-average molecular weights:

$$\bar{M}_1 = \bar{M}_{\mathrm{n}} = M^{\mathrm{M}}\,\mathrm{DP} = M^{\mathrm{M}}(1 - \beta)^{-1},$$

This leads to

$$\mathrm{DP} = (1 - \beta)^{-1}; \quad \beta = (\mathrm{DP} - 1)/\mathrm{DP}, \quad (11a)$$

$$\bar{M}_2 = \bar{M}_{\mathrm{w}} = M^{\mathrm{M}}(1 + \beta)/(1 - \beta) = M^{\mathrm{M}}(2\mathrm{DP} - 1), \quad (11b)$$

$$\bar{M}_3 = \bar{M}_z = M^{\mathrm{M}}\, 2\beta(2 + \beta)/(1 - \beta^2) = M^{\mathrm{M}}[3\mathrm{DP} - \tfrac{5}{2} - 1/2(2\mathrm{DP} - 1)]. \quad (11c)$$

Note that for DP reasonably large this gives the expected 1 : 2 : 3 ratio of the most probable Schulz distribution (e.g. if DP = 10 the ratio is 1 : 1.9 : 2.72; if DP = 50 the ratio is 1 : 1.98 : 2.945; etc., improving as DP increases).

3. Double-stranded polymers

A molecule like DNA can be represented as a pair of chains of joined monomer units, linked by a number of hydrogen-bonded base–base interactions. Initially we will suppose that as well as the species I, species like II, III and IV (fig. 1) are accessible to the degradation process.

We suppose, moreover, that the two strands of the molecule are distinguishable, as are the head and tail ends. This most unsymmetrical representation is chosen so as to remove complicating statistical weight factors in the entropy (and hence free energy) terms which arise from symmetry. Naturally the final result of the analysis of a more symmetrical model will be equivalent. Any particular species may be referred to by the triad of integers (j, k, l) where $j > 0$ is the length of the first strand, $k > 0$ is the length of the second strand, and l is the offset between the tail ends

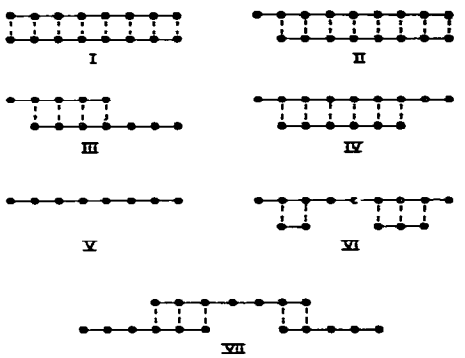

Fig. 1. Topological models for some DNA structures that may be accessible to the ultrasonic degradation process. – – – represent hydrogen bonding between base pairs.

of the two strands ($-k < l < j$), i.e. if $l \geqslant 0$ there is a hydrogen bond from the $(l+1)$th base residue from the tail of the j-strand to the tail end residue on the k-strand, while if $l \leqslant 0$ the linkage is from the $(1-l)$-th residue on the k-strand to the tail residue on the j-strand.

The assumption is now made that the standard free energy of the species (j, k, l) is given by

$$G^0_{jkl} = a(T) + b(T)n + g(T)m, \tag{12}$$

where n is the number of monomer units in the species ($n = j + k$), and m is the number of hydrogen-bonded base linkages. (If l is positive m is the lesser of $j - l$ and k, and if l is negative m is the lesser of $k + l$ and j.) Following the argument previously used for the simple polymer, we obtain:

$$\mu^M_{jkl} = a(T)/n + b(T) + g(T)m/n + (RT/n)\ln C_{jkl}, \tag{13}$$

and so

$$C_{jkl} = e^{[nd(T) - a(T) - mg(T)]/RT}. \tag{14}$$

A detailed analysis of the possible species shows that there are $(n-1)^2$ possible structures for an n-mer, since it may be represented $(j, n-j, l)$ where $1 \leqslant j \leqslant n-1$ and $j - n < l < j$ (i.e. each of the two parameters may independently take one of $(n-1)$ values). Moreover, of these, exactly $4(n-2m)$ will have m hydrogen bonded linkages, except when $m = \frac{1}{2}n$ for which there will be a single species. Thus

$$C_{nm} = 4(n-2m)e^{[nd(T) - a(T) - mg(T)]/RT} + \delta_{n,2m}\, e^{[nd(T) - a(T) - ng(T)/2]/RT}, \tag{15}$$

$$C_n = \sum_{m=1}^{(n-1)/2} 4(n-2m)\, e^{[dn(T) - a(T) - mg(T)]/RT}, \quad \text{for } n \text{ odd}, \tag{16a}$$

$$C_n = \sum_{m=1}^{n/2-1} 4(n-2m)\, e^{[nd(T) - a(T) - mg(T)]/RT} + e^{[nd(T) - a(T) - ng(T)/2]/RT}, \quad \text{for } n \text{ even}. \tag{16b}$$

Here, $\delta_{n,2m}$ is the Kronecker delta; it takes the value 1 if $n = 2m$, zero otherwise. Writing $\gamma = e^{-g(T)/RT}$, $\eta = e^{d(T)/RT}$, $k = e^{-a(T)/RT}$ the sum simplifies to

$$C_n = [k\eta^n/(1-\gamma)^2]\,[(4n-8)\gamma - 4n\gamma^2 + \gamma^{n/2+2} + 6\gamma^{n/2+1} + \gamma^{n/2}], \quad \text{for } n \text{ even}, \tag{17a}$$

$$C_n = [k\eta^n/(1-\gamma)^2]\,[(4n-8)\gamma - 4n\gamma^2 + 4\gamma^{n/2+3/2} + 4\gamma^{n/2+1/2}, \quad \text{for } n \text{ odd}. \tag{17b}$$

These expressions enable the following sums to be determined

$$\sum C_n, \quad \sum nC_n, \quad \sum n^2 C_n, \quad \sum n^3 C_n,$$

where in general the summation index will range from 2 to infinity. Making use of the result

$$\sum_{n=0}^{\infty} n^j y^n = (y\,\mathrm{d}/\mathrm{d}y)^j (1-y)^{-1},$$

the following sums are obtained (after much tedious manipulation)

$$\sum_{n=2}^{\infty} C_n = \frac{k\eta^2\gamma(1+\eta)^2}{(1-\eta)^2(1-\eta^2\gamma)}, \tag{18a}$$

$$\sum_{n=2}^{\infty} nC_n = C = \frac{2k\eta^2\gamma(1+\eta)(1+2\eta-\eta^2-2\eta^3\gamma)}{(1-\eta)^3(1-\eta^2\gamma)^2}, \tag{18b}$$

$$\sum_{n=2}^{\infty} n^2 C_n = \frac{4k\eta^2\gamma}{(1-\eta)^4(1-\eta^2\gamma)^3}$$
$$\times (1+5\eta+2\eta^2-3\eta^3+\eta^4+\eta^2\gamma-6\eta^3\gamma$$
$$-10\eta^4\gamma+2\eta^5\gamma+\eta^6\gamma+\eta^5\gamma^2+4\eta^6\gamma^2+\eta^7\gamma^2), \quad (18c)$$

$$\sum_{n=2}^{\infty} n^3 C_n = \frac{4k\eta^2\gamma}{(1-\eta)^5(1-\eta^2\gamma)^4}$$
$$\times (2+17\eta+13\eta^2-15\eta^3+9\eta^4-2\eta^5+8\eta^2\gamma$$
$$-23\eta^3\gamma-85\eta^4\gamma+19\eta^5\gamma+17\eta^6\gamma-8\eta^7\gamma$$
$$+2\eta^4\gamma^2-5\eta^5\gamma^2+59\eta^6\gamma^2+31\eta^7\gamma^2-13\eta^8\gamma^2$$
$$-2\eta^9\gamma^2-\gamma^7\gamma^3-11\eta^8\gamma^3-11\eta^9\gamma^3-\eta^{10}\gamma^3). \quad (18d)$$

At this point it is noted that γ appears always as an essential part of the quantity $\eta^2\gamma$. It is also noted that convergence criteria in the sums evaluated, and the definitions of η and γ require $0<\eta<1$; $0<\eta^2\gamma<1$. We change the parameterization to remove γ from the expressions and include $\alpha = 1-\eta^2\gamma$, and form the expressions for the moments of the distribution:

$$\bar{M}_n = M^M 2(1-\eta^2+2\eta\alpha)[\alpha(1-\eta^2)]^{-1}, \quad (19a)$$

$$\bar{M}_w = M^M 2[2(1-\eta^2)^2-\alpha(1-\eta^2)(1-\eta^2-4\eta)$$
$$+\alpha^2\eta(1+4\eta+\eta^2)][\alpha(1-\eta^2)(1-\eta^2+2\eta\alpha)]^{-1}, \quad (19b)$$

$$\bar{M}_z = M^M [12(1-\eta)^3(1+\eta)^2$$
$$-12\alpha(1-\eta)^2(1+\eta)(1-2\eta-\eta^2)$$
$$+\alpha^2(1-\eta)(1-3\eta+10\eta^2+9\eta^3+\eta^4)$$
$$+\alpha^3\eta(1+\eta)(1+10\eta+\eta^2)]$$
$$\times \{\alpha(1-\eta)[2(1-\eta^2)^2-\alpha(1-\eta^2)(1-\eta^2-4\eta)$$
$$+\alpha^2\eta(1+4\eta+\eta^2)]\}^{-1}. \quad (19c)$$

4. Relationship between DNA molecular weight distribution and unpaired bases

According to the model there will be associated with the degradation some unpairing of the base linkages at the ends of the fragments. It is necessary to suppose, however, that the regime is such that this unpairing is fairly limited (else the model breaks down in that fully denatured single-strand species have not been considered in the statistics). A parameter expressing the degree of this unpairing will be a useful feature in the model, particularly as it may be experimentally accessible through hyperchromicity measurements. We define the parameter H as the number of unpaired bases/total number of bases, and proceed to calculate H via the expressions for C_{jkl} [eq. (14)].

When there are n bases and m hydrogen bonded linkages, there will always be $n-2m$ unpaired bases in the molecule exactly. Thus the concentration of unpaired bases is given by

$$\sum_n \sum_m (n-2m)C_{nm} = \frac{4k(1-\alpha)\eta(1+\eta)}{\alpha(1-\eta)^3}, \quad (20)$$

the total base concentration by

$$\sum_n \sum_m nC_m = \sum_n nC_n$$
$$= \frac{2k(1-\alpha)(1+\eta)(1-\eta^2+2\eta\alpha)}{\alpha^2(1-\eta)^3} \quad (21)$$

and the required useful measure of the degree of base unpairing by

$$H = 2\alpha\eta/(1-\eta^2+2\alpha\eta). \quad (22)$$

At this point we will rewrite the other expressions, replacing the thermodynamic parameters k, η, and α, with the more experimentally accessible H, C ($=\Sigma nC_n$) and D ($=\bar{M}_n/M^M$). The resulting formulae are:

$$\Sigma C_n = C/D, \quad (23a)$$

$$\Sigma nC_n = C, \quad (23b)$$

$$\sum n^2 C_n = C\{D[2 - 2H + H^2 + \tfrac{1}{2}H(H^2 + 4/D^2)^{1/2}] + 2H - 2\}, \quad (23c)$$

$$\sum n^3 C_n = 3C\{D^2[2 - 4H + 3H^2 - \tfrac{1}{2}H^3 + \tfrac{1}{2}H(H + 4/D^2)^{1/2}] - D(4 - 6H + 2H^2) + \tfrac{4}{3} - H\}, \quad (23d)$$

$$\bar{M}_n = M^M D, \quad (24a)$$

$$\bar{M}_w = M^M\{D[2 - 2H + H^2 + \tfrac{1}{2}H(H^2 + 4/D^2)^{1/2}] + 2H - 2\}, \quad (24b)$$

$$\bar{M}_z = 3M^M\{D^2[2 - 4H + 3H^2 - \tfrac{1}{2}H^3 + \tfrac{1}{2}H(H^2 + 4/D^2)^{1/2}] - D(4 - 6H + 2H^2) + \tfrac{4}{3} - H\} \times \{D[2 - 2H + H^2 + \tfrac{1}{2}H(H + 4/D^2)^{1/2}] + 2H - 2\}^{-1}. \quad (24c)$$

In the region of interest $D \gg 1$ (i.e. the fragments are still medium-sized polymers) only the terms in the leading power of D need be considered. Eqs. (24) therefore simplify to:

$$\bar{M}_n = M^M D, \quad (25a)$$

$$\bar{M}_w = 2M^M D(1 - H + \tfrac{3}{4}H^2), \quad (25b)$$

$$\bar{M}_z = 3M^M D(4 - 8H + 7H^2 - H^3)/(4 - 4H + 3H^2) = 3M^M D[1 - H + 2H^3/(4 - 4H + 3H^2)]. \quad (25c)$$

These results clearly show the expected 1 : 2 : 3 ratio, decreasing significantly with base unpairing.

5. Discussion

The thermodynamic theory presented predicts that extensively sonicated polymers (i.e. those sonicated continuously to a limiting molecular weight) would have a molecular weight distribution corresponding to that of the most probable Schulz distribution. Since polymers which have been synthesized randomly or degraded randomly are known to have this molecular weight distribution [14,16], the thermodynamic model used to describe the equilibrium molecular weight distribution appears soundly based.

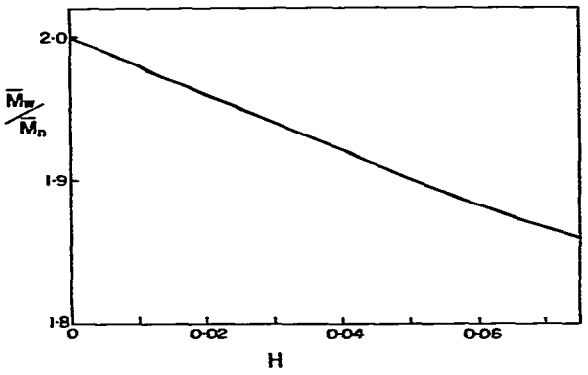

Fig. 2. Relationship predicted [eq. (24)] between the fraction of unpaired bases present in the DNA sonication products (H), and the degree of polydispersity.

Extension of the theory to double-stranded polymers such as DNA reveals several interesting and previously unknown aspects about extensively sonicated DNA. The molecular weight distribution of such DNA will depend on the extent to which hydrogen bond linkages are removed in the sonication process. A distribution which is significantly sharper than the most probable Schulz distribution will be achieved in association with only a very small degree of base unpairing at the fragment termini [eqs. (25)]. While there is compelling evidence that no extensive denaturation occurs in conjunction with sonic degradation under appropriate experimental conditions [6,8, 10,14], it is also apparent that the small amount of unpairing required is not experimentally ruled out, and that a measurable amount of denaturation does occur after a long period of sonication [10,14].

The theory in its present form, like all theories based on equilibrium thermodynamics, has nothing to say about the rate of the degradation, the relation to starting material, nor the distribution of fragments after short periods of sonication. It does however predict a clear relationship between the amount of base unpairing and the sharpness of the distribution (represented by the degree of polydispersity $\bar{M}_w/\bar{M}_n$ of fragments at equilibrium — fig. 2). It may well be possible to vary these quantities with varying experimental conditions, but the essential relationship should hold.

The theory as developed here does not take single-strand fragments properly into account. It is concern-

ed primarily with the regime where denaturation is minimal, and unpaired bases appear only at the "jagged ends" of essentially double-strand DNA molecules. As well as the single-strand species V, species like VI and VII (fig. 1) are not taken into account in the statistics.

Clearly, at very high levels of the extent of DNA denaturation, the molecular weight distribution of the essentially single-stranded DNA will again approach that of the most probable Schulz distribution. If the DNA is totally denatured, and of relatively high molecular weight, the distribution would be described by $\bar{M}_n : \bar{M}_w : \bar{M}_z = 1 : 2 : 3$ etc., the same as predicted for the "equilibrium" distribution of other single-strand polymers.

This theory also implicitly predicts that the "equilibrium" molecular weight distribution of native DNA will be independent of the initial molecular weight of the DNA and its tertiary structure (i.e. whether the DNA is initially rod-like, random coil, circular or supercoiled). In contrast, however, the *kinetics* of the degradation process do appear to depend on the rigidity of the DNA [14]. The molecular weight distribution could, however, be influenced by the presence of other compounds which interact with DNA (e.g. metal ions, drugs, dyes, etc.) since the local susceptibility to bond cleavage could be altered by these agents if, and only if, they were not randomly distributed along the DNA helix. Other substances in the working solution that could influence the course of a high-temperature reaction may also have a marked effect on the position and nature of the equilibrium (e.g. free-radical scavengers).

Experiments are currently in progress to examine these predictions by evaluating directly the molecular weight distribution of DNA at its sonication induced limiting molecular weight, under different experimental conditions.

Acknowledgement

This work was supported by the Australian Research Grants Committee.

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