BBA 66549

A NEW APPROACH TO THE ELUCIDATION OF THE INITIAL KINETICS OF NEUTRAL AND ACID DEOXYRIBONUCLEASES

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(Received October 8th, 1971)

SUMMARY

The initial kinetics of the enzymatic degradation of fully deproteinized native DNA by either beef pancreas neutral deoxyribonuclease (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.5) or hog spleen acid deoxyribonuclease (deoxyribonucleate 3'-nucleotidohydrolase, EC 3.1.4.6.) were thoroughly investigated, using analytical ultracentrifugation to determine the ratio of single-strand breaks to double-strand breaks.

Beef pancreas deoxyribonuclease degraded DNA in a non-random manner and behaved as a region-specific enzyme.

For hog spleen deoxyribonuclease, the independence of haplotomic (formation of single-strand breaks) and diplotomic (formation of double-strand breaks) mechanisms was confirmed, though predominance of the former could be demonstrated.

INTRODUCTION

Since the well-documented reviews of Laskowski[1] and Bernardi[2] only a few new data have added to our knowledge of the behaviour of deoxyribonuclease I (deoxyribonucleate oligonucleotidohydrolase (EC 3.1.4.5), or neutral deoxyribonuclease) and deoxyribonuclease II (deoxyribonucleate 3'-nucleotidohydrolase (EC 3.1.4.6), or acid deoxyribonuclease) and none to that of their specificity during the early stages of DNA degradation.

For deoxyribonuclease I, some interesting results have been obtained by Melgar and Goldthwait[3], who showed the influence of the ionic environment on the depolymerization mechanism of DNA, deduced from a treatment of viscosimetric results according the method of Cavalieri and Rosenberg[4]. For deoxyribonuclease II, the main results concern its action on the transforming ability of *Haemophilus influenzae* DNA[5], the possibility[6-8] or impossibility[9] of the elimination of its non-specific phosphodiesterase activity and, of great interest, the indirect evidence for allosteric behaviour in the presence of a protein inhibitor[10].

Despite the information so far obtained our understanding of the kind of specificity, except for structural requirements, that deoxyribonuclease I and deoxyribonuclease II are able to exhibit during the early stages of DNA degradation remains poor.

This fact seems undoubtedly due to the difficulties encountered in the preparation of great quantities of homogeneous and monodisperse substrates, showing no contamination by RNA and proteins. The knowledge of properties such as the base composition or the nearest neighbour frequencies of the DNA samples submitted to degradation, or the analysis of the terminal nucleotides of fragments present in limit digests of DNA after a hydrolysis by deoxyribonuclease I[11] or deoxyribonuclease II[12,13], do not give information about a hypothetic initial specificity.

During a recent and promising attempt[14], using the electrochemical properties of the nucleic bases to elucidate the nature of the termini resulting from a limited action of deoxyribonuclease I on various DNAs, we used analytical ultracentrifugation to estimate the number of single-strand and double-strand breaks resulting from the enzymatic attack. Significant differences between our results and those quoted by Thomas[15] and Young and Sinsheimer[16] drove us to question the generally considered lack of specificity of deoxyribonuclease I towards DNA, at least during its early action. A similar study carried out with deoxyribonuclease II as a degradative agent confirmed the independence of its haplotomic and diplotomic mechanisms, *i.e.* the mechanisms resulting in single-strand and double-strand breaks, respectively, without establishing the generally postulated predominance of the latter[2,5].

MATERIALS AND METHODS

Biochemicals and chemicals

Fresh calf thymus was obtained locally, immediatedly after slaughtering.

Micrococcus lysodeikticus dried cells were purchased from Miles Laboratories. *Proteus vulgaris* frozen cells were prepared in the Laboratoire de Chimie Bactérienne du C.N.R.S. (13-Marseille). *Escherichia coli* B/r was cultivated in our laboratory.

Pronase and beef pancreas ribonuclease (1 ×) were purchased from Calbiochem and Worthington, respectively. Lysozyme was a Serlabo product. Sodium dodecylsulfate was a Sigma product. Beef pancreas deoxyribonuclease I was a ribonuclease-free electrophoretically purified product of Worthington (code DPFF OED) with a Kunitz[17] activity of 2100 units/mg.

Hog spleen deoxyribonuclease II was prepared in our laboratory, using a previously described method[9]; its specific activity was 339 (the specific activity was calculated by dividing the number of acid-soluble units of the enzyme solution by its $A_{280\ nm}$[18]; Bernardi's[2] purest preparation had a specific activity of 350).

Preparation of DNA samples

Calf thymus DNA (DNA CT) was extracted according to the method of Kay *et al.*[19], and further deproteinized by digestion with pronase (0.1 mg pronase per mg DNA, standard saline citrate (0.15 M NaCl–0.015 M trisodium citrate) 24 h at 37 °C) followed by shaking with several changes of chloroform–isoamyl alcohol (9:1, v/v). Final purification included repeated treatments of the calf thymus DNA solution with

0.2 vol. of freshly distilled, water-saturated phenol, until no solid interface between the phenolic and aqueous layers could be detected.

Bacterial DNAs were extracted from *E. coli* B/r (DNA EC) *M. lysodeikticus* (DNA ML) and *P. vulgaris* (DNA PV) cells using the method of Marmur[20] as a first purification step. Full deproteinization and RNA elimination were achieved by an alternation of ribonuclease digestions and phenol treatments, ended by isopropanol precipitation. *H. influenzae* DNA (DNA HI) was a gift of Dr M-R. Chevallier (Laboratoire de Génétique Physiologique, Strasbourg); it was purified in the same way. Thermal denaturation profiles, ultraviolet spectra and negative Lowry[21] tests indicated a very low contamination of our DNA samples, the main characteristics of which are listed in Table I.

TABLE I

MOLECULAR WEIGHTS, SPECTRAL AND MELTING CHARACTERISTICS OF THE VARIOUS DNA PREPARATIONS SUBMITTED TO DEGRADATION

DNA preparation	*Neutral*		*Alkaline*		*Ratio* $\frac{A_{260\ nm}}{A_{230\ nm}}$	*Melting profiles*		
	$s^{\circ}_{20,w}$	$M_w \times 10^{-6}$	$s^{\circ}_{20,w}$	$M_w \times 10^{-6}$		T_m (°C)*	$\Delta A_{260\ nm}$ (%)	*T.W.*(°C)**
CT 1	23.0	9.6	21.7	3.4	2.29	86.2	38.2	4.9
CT 2	23.4	10.2	22.7	3.9	2.27	86.4	39.1	5.1
PV	24.8	12.0	28.0	6.4	2.33	86.2	37.5	3.0
EC 1	25.2	12.5	26.6	5.7	2.27	89.5	39.8	2.9
EC 2	23.1	9.8	25.6	5.1	2.26	90.0	39.0	2.7
ML 1	24.7	11.8	27.2	6.0	2.23	98.0	38.5	2.5
ML 2	23.8	10.7	21.3	3.3	2.21	98.2	38.0	2.9
HI	20.2	6.8	16.1	1.6	2.32	85.1	41.0	3.8

* In standard saline citrate.

** Transition width calculated between 17 and 83% of the total hyperchromicity, according to Doty *et al.*[38].

Enzymatic degradation of DNA

In our reaction conditions, no hyperchromicity could be detected during enzymatic hydrolysis.

The reaction of deoxyribonuclease I was carried out at 25 °C, under previously described conditions[14] (buffer: 0.15 M NH_4Cl–0.01 M ammonium acetate–5 mM $MgCl_2$, pH 5.50 ± 0.02; 8 ng deoxyribonuclease I per ml of reaction medium containing 100 μg DNA). After incubation deoxyribonuclease I was inactivated by EDTA. Ultracentrifugal analysis was performed after dialysis against 1 M NaCl–1 mM EDTA; in this medium sedimentation coefficients remained constant for up to 4 days.

The reaction of deoxyribonuclease II was carried out at 25 °C, in standard saline citrate containing 2 mM EDTA and adjusted at pH 5.00 ± 0.02; the concentrations were 100 μg/ml for DNA and 3–9 ng/ml for deoxyribonuclease II. After incubation deoxyribonuclease II was inactivated either by increasing the ionic strength (final concentration: 1 M NaCl–20 mM $MgCl_2$) or by shaking with chloroform–isoamyl alcohol (9:1, v/v); in both cases results were identical.

Physical methods

pH values were measured with a Radiometer PHM 4d pH meter.

Spectrophotometric and colorimetric determinations were carried out on a Zeiss PMQ II spectrophotometer.

Melting curves of DNA were recorded on a Perkin–Elmer 402 spectrophotometer, equipped with a thermostated cell TU-137 and a scale expansion device permitting analysis of DNA solutions with $0.25 < A_{260\ nm} < 0.30$. The accuracy of the T_m determinations was $\pm$ 0.4 °C.

Light-scattering determinations were performed on a Sofica photogoniodiffusometer, thermostated at 25 $\pm$ 0.2 °C, at six preselected angular values between 30 and 90 °C. The cell contained 22 ml of DNA solution at 70 μg/ml and 80 ng of deoxyribonuclease II. In these conditions, the drop of the weight-average molecular weight (M_w), deduced from the graphic representation of Zimm[22], did not exceed 30–40% for a 2-h incubation, thus permitting analysis of the mechanism of initial degradation according to the method of Cavalieri *et al.*[4].

Sedimentation runs were performed in the Spinco Model E ultracentrifuge, equipped with an ultraviolet light source and a monochromator. Boundary sedimentation was performed in 12-mm or 30-mm Kel F centerpieces. As described by Studier[23], the solvent was 1.0 M NaCl for neutral sedimentations and 0.9 M NaCl–0.1 M NaOH for alkaline sedimentations. The extrapolation of $s_{20,w}$ at infinite dilution was obtained by sedimentation of four dilutions of the same sample with $0.20 < A_{260\ nm} < 0.90$. Though Hearst *et al.*[24] have shown the effect of concentration on sedimentation coefficients of high molecular weight DNA, the size of our samples caused this extrapolation to be unnecessary in most cases, either in neutral or in alkaline medium; this fact was in agreement with the results of Londos-Gagliardi *et al.*[25]. The distribution function of sedimentation coefficients was computed according to the method of Shumaker and Schachman[26].

RESULTS

Estimate of the number of single-strand and double-strand breaks produced in native DNA by enzyme action

The drop of DNA molecular weight as a function of the degradation time was estimated from the variation of its sedimentation coefficients, either in neutral or in alkaline medium, using Studier's[23] equations:

Neutral medium (1.0 M NaCl):

$$s^{\circ}_{20,w} = 0.0882\ M^{0.346} \quad (1)$$

Alkaline medium (0.9 M NaCl–0.1 M NaOH):

$$s^{\circ}_{20,w} = 0.0528\ M^{0.400} \quad (2)$$

Though derived for homogeneous λ phage DNA, these equations are in good agreement with the results obtained by Doty *et al.*[27], when relating $s^0_{20,w}$ to M_w. We have used this fact to introduce our molecular weight values as weight-average parameters in the series development described by Charlesby[28], derived for the degradation of polydisperse long-chain polymers, such as preparations obtained by mechanical rupture of large-sized chromosomes, and characterized by:

$$M_n = \tfrac{1}{2} M_w = \tfrac{1}{3} M_z$$

Using the first two terms of Charlesby's[28] development, we obtained a simple equation:

$$\frac{(M_w)_t}{(M_w)_0} = \frac{1}{1 + \frac{1}{2} P \cdot U_w} \tag{3}$$

where P is the probability of rupture between two successive monomers, U_w the weight-average degree of polymerization of the degraded polymer at time t, $(M_w)_0$ and $(M_w)_t$ the values of the weight-average molecular weight at time 0 and t, respectively. As $P \cdot U_w$ can be considered as the weight-average number of single-strand breaks (alkaline medium) or of double-strand breaks (neutral medium) at time t, a combination of Eqn 3 with either Eqn 1 or Eqn 2 affords a direct estimate of the weight-average number of double-strand breaks (d_w) or of single-strand breaks (s_w):

Neutral medium:

$$d_w = 2 \left[\left(\frac{(s^0_{20,w})_0}{(s^0_{20,w})_t} \right)^{2.89} - 1 \right] \tag{4}$$

$(s^0_{20,w})_0$ and $(s^0_{20,w})_t$ are sedimentation coefficients in 1.0 M NaCl, at times 0 and t, respectively.

Alkaline medium:

$$s_w = 4 \left[\left(\frac{(s^0_{20,w})_0}{(s^0_{20,w})_t} \right)^{2.50} - 1 \right]$$

$(s^0_{20,w})_0$ and $(s^0_{20,w})_t$ are sedimentation coefficients in 0.9 M NaCl–0.1 M NaOH, at times 0 and t, respectively.

Both d_w and s_w refer to each parent DNA molecule. When DNA samples presented some single-strand breaks before being submitted to degradation, the value of s_w was determined using a theoretical $(s^0_{20,w})$ corresponding to 0.5 neutral $(M_w)_0$.

The ratio R of single-strand to double-strand breaks was used to determine the relative importance of haplotomic and diplotomic mechanisms:

$$R = \frac{s_w}{d_w}$$

Degradation of native DNA by deoxyribonuclease I

The four DNAs submitted to the action of deoxyribonuclease I had similar molecular weight repartitions as judged from the distribution curves of their sedimentation coefficients ($g(s)$ curves, Fig. 1).

In our reaction conditions, no lag period could be evidenced in the decrease of neutral molecular weights, except for DNA ML 1; this poorer reactivity of the DNA of *M. lysodeikticus* towards deoxyribonuclease I had already been noticed during a viscosimetric and electrochemical study of its degradation[14].

For each DNA, the increase of s_w and d_w was a linear function of the incubation time; moreover, the respective values of s_w and d_w obtained for the various DNAs at identical degradation times, were close to one another (Table II; Fig. 2). This zero order behaviour of the hydrolysis resulted in the constancy of the ratio R which, in our experiments, remained between 22 and 32. From the various sets of R values cor-

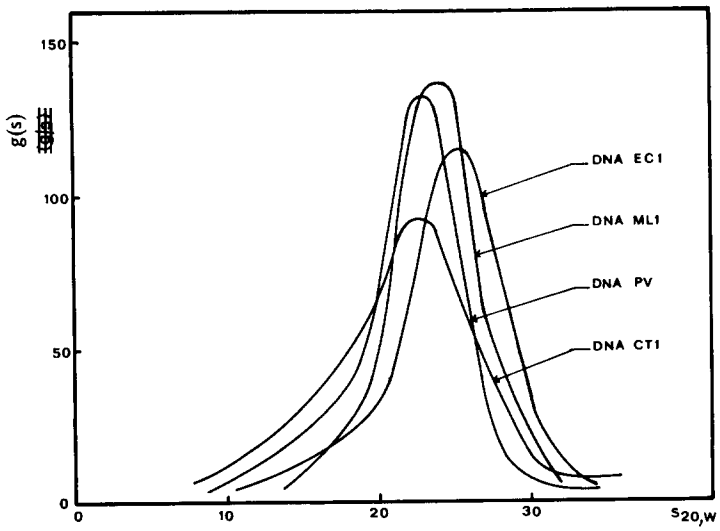

Fig. 1. Repartition curves of neutral sedimentation coefficients. The function $g(s)^*$ was computed for the native DNAs submitted to the action of deoxyribonuclease I. Sedimentation was performed as described under Materials and Methods: 1.0 M NaCl; 15 μg DNA/ml; 20 °C; 44 000 rev./min. DNA CT 1: calf thymus DNA; DNA PV: *P. vulgaris* DNA; DNA ML 1: *M. lysodeikticus* DNA; DNA EC 1: *E. coli* DNA.

TABLE II

DEGRADATION OF NATIVE DNA BY DEOXYRIBONUCLEASE I

The number of single-strand breaks (s_w) and of double-strand breaks (d_w) computed for each incubation time refers to the undegraded parent molecule.

Incubation time (min)	*DNA CT 1*		*DNA PV*		*DNA EC 1*		*DNA ML 1*	
	s_w	d_w	s_w	d_w	s_w	d_w	s_w	d_w
0	1.64	0	0	0	0.38	0	0	0
5	8.00	0.34	4.48	0.10	9.16	0.23	4.60	0
10	13.44	0.43	16.60	0.66	21.00	0.69	6.00	0.29
15	21.20	1.00	23.60	1.08	27.70	0.98	—	0.43
20	30.30	1.26	37.40	1.59	—	—	15.20	0.57
30	47.80	1.92	45.60	2.00	61.80	2.24	32.90	1.19
45	64.60	2.36	78.40	3.34	82.00	2.64	47.20	1.52
60	89.60	3.44	95.20	3.72	92.00	3.82	68.80	2.72

responding to the degradion of the four DNA samples, the following regression lines could be drawn (Fig. 2):

DNA CT 1 : $R = 0.02\,t + 24.87$

DNA PV : $R = 0.03\,t + 22.80$

DNA ML 1 : $R = 0.09\,t + 23.15$

DNA EC 1 : $R = -0.07\,t + 30.60$

(except for DNA CT 1, 5-min values were neglected in the calculation of the regression parameters, because of an important deviation of R resulting from too low a variation in neutral M_w).

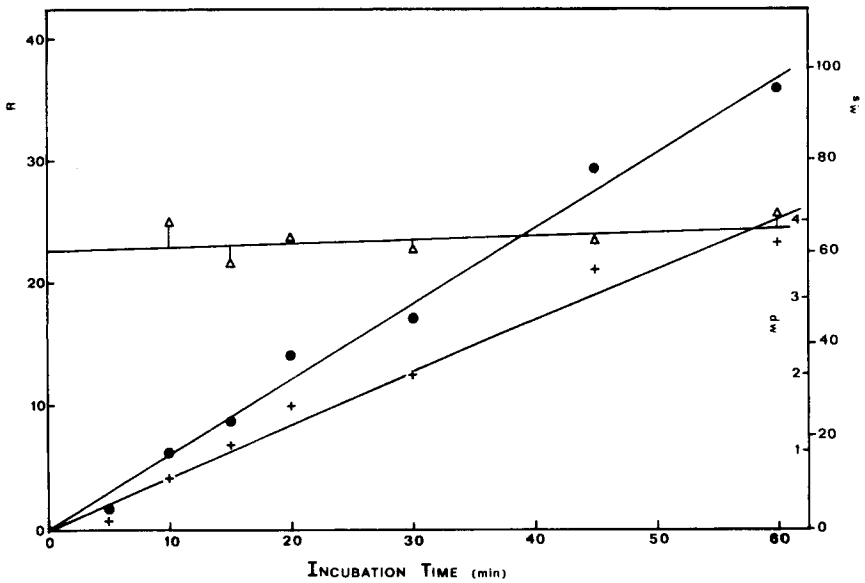

Fig. 2. Degradation of native *P. vulgaris* DNA (DNA PV, Table II) by deoxyribonuclease I, as a function of the incubation time. The incubation was performed as described under Materials and Methods. Eqns 4 and 5 were used to determine the respective weight-average numbers of double-strand breaks (d_w, +—+) and of single-strand breaks (s_w, ●—●), from the neutral and alkaline sedimentation coefficients. The ratio $R = s_w/d_w$ is plotted in the form of the regression line: $R = 0.03\,t + 22.80$ (△—△).

TABLE III

DEGRADATION OF NATIVE DNA BY DEOXYRIBONUCLEASE II

Number of single-strand breaks (s_w), double-strand breaks (d_w) and corresponding values of R obtained in various conditions of degradation. DNA CT 2: 8 ng deoxyribonuclease II/100 μg DNA; DNA ML 2 (a): 8 ng deoxyribonuclease II/100 μg DNA; DNA ML 2 (b): 3 ng deoxyribonuclease II/100 μg DNA.

Incubation time (min)	*DNA CT 2*			*DNA ML 2 (a)*			*DNA ML 2 (b)*		
	s_w	d_w	R	s_w	d_w	R	s_w	d_w	R
5	9.8	0.28	35.0	16.6	0.14	118	2.7	0	—
10	21.8	0.70	31.1	32.6	0.60	54	7.4	0	—
15	31.4	1.26	25.0	52.8	1.44	37	16.8	0.32	52
20	36.0	2.30	15.6	58.6	2.00	29.3	—	—	—
25	—	—	—	76.0	2.30	33	—	—	—
30	41.6	2.50	16.6	80.0	3.80	21	25.5	0.82	31.1
45				—	—	—	43.6	1.48	29.4
60				91.6	4.20	21	51.2	1.44	35.7
90							65.2	1.65	40.5
120							78.0	2.78	28
180							92.0	4.85	19

Degradation of native DNA by deoxyribonuclease II

A different series of DNA samples was used with deoxyribonuclease II, though arising from the same preparations as for deoxyribonuclease I; non-simultaneous

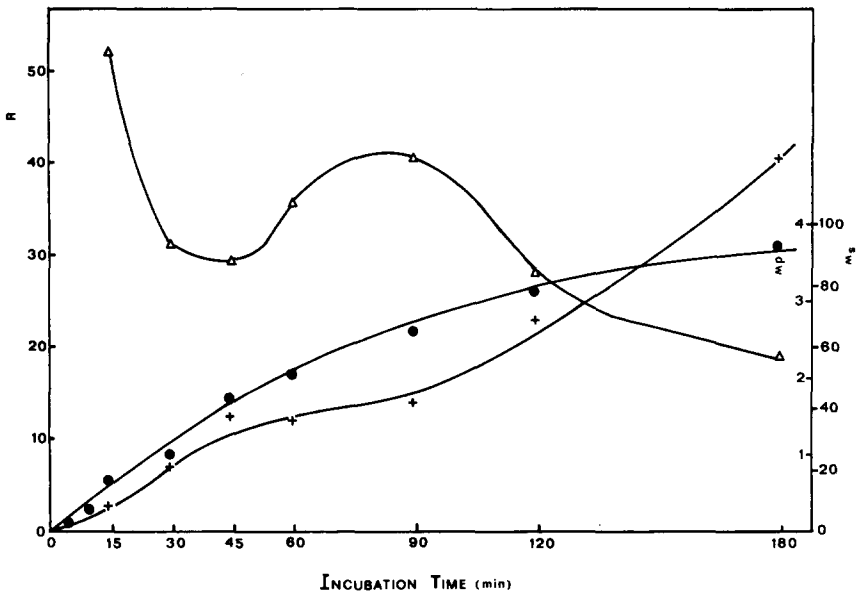

Fig. 3. Degradation of native *M. lysodeikticus* DNA (DNA ML 2 (b), Table IV) by deoxyribonuclease II as a function of the incubation time. The incubation was performed as described under Materials and Methods. Eqns 4 and 5 were used to determine the respective weight-average numbers of double-strand breaks (d_w, +—+) and of single-strand breaks (s_w, ●—●) as well as their ratio $R = s_w/d_w$ (△—△), from the neutral and alkaline sedimentation coefficients.

TABLE IV

INITIAL DEGRADATION OF *E. coli* DNA BY DEOXYRIBONUCLEASE II

3 ng deoxyribonuclease/100 μg DNA EC 2 per ml.

Incubation time (min)	$s^\circ_{20,w}$ *(neutral)*	$s^\circ_{20,w}$ *(alkaline)*	s_w	d_w
0	22.12	24.20	0	0
2	22.22	22.50	0.56	0
4	21.72	21.00	1.40	0
6	22.12	20.15	2.04	0
8	22.40	17.90	4.06	0
10	22.10	16.50	5.90	0
15	22.41	14.80	9.12	0
20	21.03	13.40	12.6	0.3

deproteinization was the only reason for using two different series. $g(s)$ values of this second series were as in Fig. 1.

No linear variation of s_w and d_w was observed in the action of deoxyribonuclease II on native DNA. At the beginning of hydrolysis, s_w increased linearly with the time; a brief lag time occured with the lowest deoxyribonuclease II concentration, then the reaction rate decreased and a zero order behaviour could no longer be observed (Table III, Fig. 3). DNA ML was slightly more reactive than other DNAs towards deoxyribonuclease II but not to deoxyribonuclease I. As no deactivation could be evidenced for deoxyribonuclease II incubated alone in the same conditions of medium

TABLE V

NUMBER OF SINGLE-STRAND BREAKS (s_w) INDUCING A 50% DECREASE OF THE NEUTRAL WEIGHT-AVERAGE MOLECULAR WEIGHT (M_w) OF VARIOUS NATIVE DNA SAMPLES DEGRADED BY DEOXYRIBONUCLEASE I

DNA preparation	M_w	s_w
CT 1	9.6	52.0
PV	12.0	45.6
EC 1	12.5	57.0
ML 1	11.8	51.0

and concentration, for up to 6 h, this decreased rate was attributed to the disappearance of the most reactive nucleotide sequences, whose limited number confirmed the initial narrow specificity of the enzyme. The case of d_w was more complicated: when a 8ng/100 μg enzyme/substrate ratio was used (Table III, DNA ML 2 (a)), no lag time occured in the decrease of neutral M_w; d_w and s_w variations did not parallel each other, so that a continuous variation of R was observed. The mutual independence of haplotomic and diplotomic mechanisms was thus confirmed by the variation of their relative importance as a function of time. When the enzyme/substrate ratio was lowered to 3 ng/100 μg (Table III, DNA ML 2 (b); Table IV), d_w presented a lag period of 10–15 min with DNA ML 2 and DNA EC 2, then it decreased in a non-regular way and R underwent fluctuations, with a final tendency to decrease (Fig. 3).

In order to ascertain these results qualitatively, light scattering was used to follow the decrease of M_w at the beginning of the degradation. The M_w values obtained from sedimentation or light scattering were not submitted to direct comparisons, since an underestimate of M_w, when computed from scattering at angular values above 30 °C[29,30] is possible. The occurence of a lag period in the decrease of M_w was confirmed for the degradation of all substrates. In the case of DNA ML 2, it was about 10 min; for DNA HI it exceeded 20 min. The apparent number of strands n, calculated from the equation of Cavalieri *et al.*[4]:

$$\log \frac{1-r}{r} = n \log t + K$$

(where $r = (M_w)_t/(M_w)_0$; t = incubation time; K = experimental constant) was 1.16 for DNA ML 2 (Fig. 4) and 1.28 for DNA HI. These values indicated a strong predominance of the diplotomic mechanism and that DNA behaved mainly as a single-stranded polymer. At the end of each series of light-scattering measurements, deoxyribonuclease II was inactivated and s_w determined by alkaline sedimentation: it was 25 and 18 for DNA ML2 and DNA HI, respectively. Thus, the haplotomic mechanism certainly played a part in the early DNA degradation and was likely responsible for n values systematically higher than 1.0.

DISCUSSION

Though some restrictions expressed by Freifelder and Trumbo[31] about the use of boundary sedimentation for quantitative determinations, its sensitivity makes it a highly usable method to detect single-strand and double-strand breaks resulting from enzymatic hydrolysis of DNA.

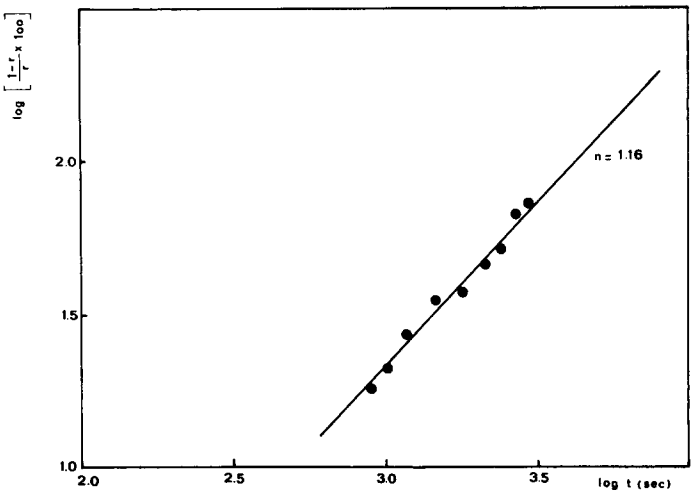

Fig. 4. Light-scattering study of the degradation of native *M. lysodeikticus* DNA (DNA ML 2) by deoxyribonuclease II. Degradation was performed as described under Materials and Methods. The apparent number of strands of the DNA sample: $n = 1.16$ was computed according to Cavalieri and Rosenberg[4], as the slope of the curve: $\log [(1-r)/r] \times 100 = n \log t + K$, where $r = (M_w)_t/(M_w)_0$.

If we consider the initial action of deoxyribonuclease I on DNA, the similar increase of s_w and d_w confirms the interdependence of haplotomic and diplotomic mechanisms, the latter being a consequence of the former. The number of identical sites, corresponding to the formation of initial single-strand breaks, is high even if, as in our experiments, no more than $3^0/_{00}$ of all internucleotide bonds were concerned in the formation of single-strand breaks during 1 h.

When comparing our results with previously published data, it must be pointed out that some of the latter refer to Z-average values (Thomas[15]), whereas others are expressed in a heterogeneous system relating Z-average numbers of scissions to M_w values (Bernardi *et al.*[5], Bach[32]). For Thomas[15], a Z-average number of 200 single-strand breaks ($s_w = 133$) was necessary to halve a calf thymus DNA molecule with $M_Z = 9.9 \cdot 10^6$ ($M_w = 6.6 \cdot 10^6$). In our series of experiments, a lower number of single-strand breaks induced the same effect in longer DNA molecules (Table V). The apparent discrepancies of these data may be explained as the result of methodological difference. In their experiments, Thomas and co-workers[33] used a lyophilized DNA with a protein content of 1.2% deduced from biuret assay; it is now well-established that freezing and thawing can induce structural alterations in DNA[34]; the biuret assay is also far less sensitive than the method of LOWRY *et al.*[21].

It is evident that not all internucleotide bonds are equally reactive towards deoxyribonuclease I, since it can only hydrolyze 25–30% of them[1]. It explains why inconsistent results are obtained if our experimental data are submitted to theoretical treatments[5,31] derived for random degradations, where all internucleotide bonds have the same probability of rupture. When computing the value of h (maximal number of nucleotide pairs which, between two single-strand breaks in opposite strands, cannot maintain the double-strand structure) from the results obtained for

the halving of DNA PV (Table V), *i.e.* $s_w = 45.6$, $d_w = 2.0$ and $U_w = 18 \cdot 10^3$ (number of nucleotide bonds per single strand) using the equation of Freifelder and Trumbo[31]:

$$h = 2 \frac{d_w U_w}{(s_w)^2} - \frac{1}{2}$$

we find 34 instead of the generally considered value of 3, corresponding to our reaction conditions. Thus, our s_w values are inconsistent with a random degradation of DNA and could reflect the occurence of a strong proportion of single-strand breaks as clusters in specific regions. Such a clustering was already indirectly observed by Melgar and Goldthwait[3] in degradation kinetics of deoxyribonuclease I, where DNA behaved as if only built from one strand. The clustering of single-strand breaks can be considered as indicative of a deoxyribonuclease I initial specificity of the same kind as the region specificity postulated by Johnson and Laskowski[35] for mung bean nuclease I. As far as this clustering reflects an increased affinity of DNA for deoxyribonuclease I, the specific regions concerned in the initial degradation must present a strong affinity for proteins; this fact makes the full deproteinization of DNA a prerequisite to any thorough analysis of its interactions with enzymes. The nature of these specific regions is not well established though they are suspected to have high A–T contents[35]. This hypothesis is in agreement with polarographic results obtained during an electrochemical study of the initial degradation of DNA by deoxyribonuclease I, where the signal corresponding to the newly formed termini could be attributed to adenine[14] whereas the signal of cytosine was lacking.

For deoxyribonuclease II as for deoxyribonuclease I, significant differences appear between our results and those quoted previously[2,5,16]; its specificity seems to concern nucleotide sequences rather than regions. The important fact is that haplotomic and diplotomic actions of deoxyribonuclease II can not be dissociated. Though the dimeric structure of deoxyribonuclease II could account for an initial predominance of diplotomy, from our results it is evident that both haplotomy and diplotomy occur together, with a relative importance which could reflect the concentration of unsymmetrical and symmetrical anti-parallel sequences in DNA. In agreement with Bernardi and co-workers[13,37] we think that these sequences could have a tetranucleotide size; the relative concentration of all possible tetranucleotide sequences is low enough to account for a rapid consumption of the most reactive ones. As concerns the predominance of one degradation mechanism over the other its evaluation depends strongly on the choice of the physical method to study the action of deoxyribonuclease II.

If physicochemical methods enable us to detect specificities in the behaviour of deoxyribonucleases towards DNA, the problem of the initial specificities of these enzymes will remain until sequence analysis is performed to determine the nature of the proximal and penultimate nucleotides, adjacent to the initial breaks.

ACKNOWLEDGEMENTS

We thank Professor Ch. Sadron for his interest and helpful suggestions. The technical help of G. Serros was greatly appreciated.

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