INITIAL KINETICS OF DNA DEGRADATION BY CHICKEN CRUDE ACID DEOXYRIBONUCLEASE

Véronique Barthélémy-Clavey, Philippe J. Sicard and Geneviève Aubel-Sadron

Centre de Biophysique Moléculaire, 45 - ORLEANS 02 (France)

Received December 18, 1970

SUMMARY

The ability of the crude acid DNase extracted from chicken erythrocytes to degrade native DNA into rod-like sub-units of $5.5 \pm 0.5 \times 10^5$ daltons, has previously been attributed to a narrow specificity of the enzyme (1).

In attempting to confirm this result, we have been conducted to conclude that such a specificity does not exist. The limited DNA degradation arises from inner pH fluctuations of the reaction medium, originating in its complexity. An elimination of these fluctuations allows the degradation to proceed up to the nucleotide stage, so that limited DNA degradation must be considered as an artefact.

In native DNA, the non-random occurence of peculiar sequences, distant from 0.5 to 1×10^6 daltons, and acting as either weak points in degradations or recognition sites in enzymatic interactions, has been postulated from various studies : hydrolysis by chicken erythrocytes crude acid DNase (crude DNase II) (1, 2), extraction of calf thymus native DNA as sub-units (3), ultra-sonic degradation (4), complex formation with RNA-polymerase (5) or pancreatic RNase (6).

This report describes the main features of initial DNA degradation by crude DNase II and briefly discusses the possible role of contaminating enzymes in the limitation of this degradation.

MATERIALS AND METHODS

DNA preparations used in our work were obtained from calf thymus (CT) and chicken erythrocytes (CE) by the method of KAY et al. (7), or from Escherichia coli (EC) by the method of MARMUR (8).

Crude DNase II was obtained as a nucleo-proteic complex according to BERNARDI et al. (1), using a lysis of chicken erythrocytes by saponin. The final pH of the enzymatic preparations was 7.3.

All degradations were carried out at 37°, with a DNA concentration of 400 µg/ml, and a constant ratio of 1 ml of crude DNase II for 30 ml of DNA solution. Incubation medium was 0.15 M NaCl or 0.15 M acetate buffer (AcB). Incubation times varied from 1 to 170 hours, but, whether a few drops of toluene had initially been added or not, in no cases could a bacterial contamination be evidenced.

After incubation, the mixture was cooled at 20° for an accurate pH determination. The degradation was stopped by increasing saline concentration to 1 M ; the enzymatic nucleo-proteic complex was then eliminated by a 2 hour centrifugation at 105.000 x g, and the DNA supernatant dialysed for 24 hours against cold 1 M NaCl before being submitted to physical measurements.

The variation of DNA molecular weight (M_w) during enzymatic degradation was followed by light-scattering ; simultaneously, the sedimentation coefficient ($S_{20,w}$) was determined and, in some cases, the distribution function of sedimentation coefficients (G(S)) computed according to SCHUMAKER et al. (9). The same discrepancies as encountered in previous works (1, 2) could be noticed between M_w and M_s values computed from $S_{20,w}$ according to STUDIER (10). It must yet be recalled that M_w values exceeding 6-7 x 10^6 daltons are at the limit of validity of light scattering measurements, as pointed out by FROELICH et al. (11).

RESULTS AND DISCUSSION

When the incubation was performed in 0.15 M NaCl, with reactants adjusted at pH 5.20, a fast decrease of M_w occured during the first hours ; then the reaction proceeded more slowly, and after 48 hours of incubation, M_w reached a constant value of about 1-2 x 10^6

daltons. No changes of M_w were observed when incubation processed further (Fig. 1). Besides this variation of M_w, a strong pH increase was noticed, the value of which was rather dependent on the enzymatic preparation used (Table 1). This spontaneous pH variation was also observed when crude DNase II was incubated without added DNA, though the final pH value was slightly lower (Fig. 1), whereas no significant changes occured when DNA was incubated alone.

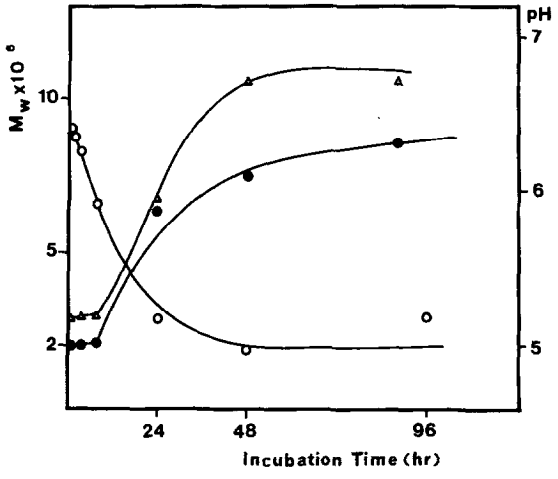

Figure 1 : M_w and pH variations during incubation of DNA CE 10 with crude DNase II in 0.15 M NaCl pH 5.20 ; o——o M_w ; Δ——Δ pH of DNA + crude DNase II ; •——• pH of crude DNase II incubated alone.

In addition to these observations, it appeared that during its initial step, DNA degradation resulted from both diplotomic and haplotomic mechanisms, as concluded from the behaviour of DNA when centrifuged in alkaline NaCl (pH 12.0) according to STUDIER (10). A mean value of one "nick" per 1-2 x 10^5 daltons could thus be determined from the ratio of the values obtained for M_s in neutral and alkaline medium, using the following expressions :

$$S_{20,w} = 0.0882\ M^{0.346} \quad \text{(neutral)}$$

$$S_{20,w} = 0.0528\ M^{0.400} \quad \text{(alkaline)}$$

Table 1

DNA degradation by various crude DNase II preparations in non-buffered medium (0.15 M NaCl, pH 5.20)

DNA sample	Incubation time	Final pH	$M_w \times 10^{-6}$	$S_{20,w}$
CT 3	0	-	7.8	21
CT 3	64	6.60	1.3	-
CT 3	98	6.92	1.2	10
CE 1	0	-	11.0	22
CE 1	24	5.14	2.5	13.8
CE 1	48	5.21	1.25	9.9
CE 1	96	5.85	1.0	6.9
CE 10	0	-	10.0	23
CE 10	48	6.71	1.8	11.4
CE 10	96	6.70	2.0	12.5

Though no differences in DNA degradation had been mentionned by BERNARDI et al. (1), when using 0.15 M AcB instead of 0.15 M NaCl as incubation medium, in our case, in the same buffered medium, we observed a fast continuous M_w decrease, so that after a 117-hour degradation the DNA samples could no more be submitted to light scattering and analytical ultracentrifugation. Simultaneously, an important formation of acid-soluble oligo-nucleotides was evidenced (Table 2). The appearance of acid-soluble products during the first hours of incubation was attributed to an auto-degradation of the enzymatic nucleoproteic complex and occured when it was incubated alone.

The fact that in our experiments, the DNA degradation resulted exclusively from the action of an acid DNase was confirmed by a series of degradation performed in 0.15 M NaCl, with initial pH values from 5.0 to 6.50. The degradation was the strongest at pH 5.0, whereas at 6.50 M_w remained unchanged. Thus, the value of the plateau observed for M_w could directly be related to the pH conditions

Table 2

DNA degradation in buffered medium (AcB pH 5.22). Samples T were incubated in the absence of enzyme. A) Degradation of DNA CT 7 ; B) Degradation of DNA EC 12.

	Incubation time (hr)	$M_w \times 10^{-6}$	$S_{20,w}$	Acid soluble degradation products %	
				DNA + enzyme	Enzyme
A)	1	6.2	18.1	0.2	0.9
	3	6.2	15	0.4	0.4
	5	3.1	12	0.8	0.7
	22	0.7	7	4.0	3.5
	45	0.16	4.5	12.3	4.6
	94	-	2	37.0	5.5
	117	-	-	52.4	5.7
	0 (T_0)	7.8	20.6	0.0	-
	117 (T_{117})	5.5	18.2	0.4	-
B)	22	0.7	7.1	4.5	3.8
	47	0.27	5	14.5	4.3
	97	-	2.9	42.4	5.2
	0 (T_0)	-	23.8	0.0	-
	97 (T_{97})	-	21.9	0.3	-

chosen for the degradation, and the possibility of action of an other neutral nucleolytic enzyme was ruled out.

Besides a hypothetical narrow enzymatic specificity, an inactivation of crude DNase II had been postulated to account for the limited degradation of DNA (12). To test this eventuality, a sample of DNA (CE. 1) was incubated for 96 hours in 0.15 M NaCl, so that M_w reached a plateau of 1.5×10^6 daltons and pH increased to 5,91. At the end of this first degradative step, half of the sample was readjusted at pH 5.20 and incubated for 74 more hours, as the unchanged half of the sample. In the acidified solution, a second degradative step occured ; M_w decreased to 7×10^5 daltons and a displacement of the G(S) curve towards the lower values was observed (Fig. 2). In the

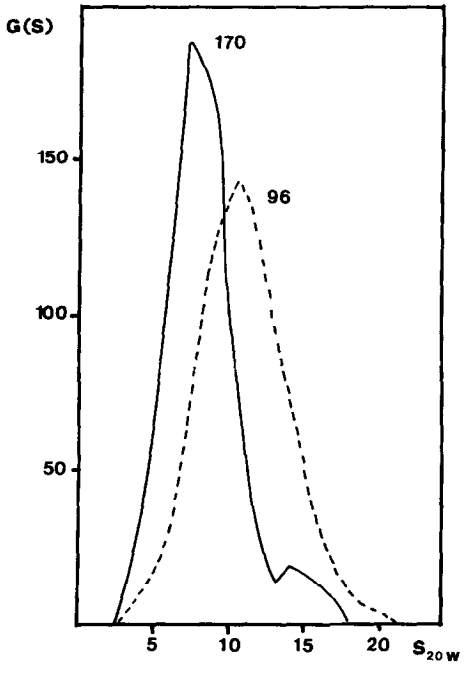

Figure 2 : G(S) curves of two samples of DNA CE 1 incubated with crude DNase II in 0.15 M NaCl pH 5.20

– – – – –: incubation during 96 hours
————: incubation during 170 hours, with a readjustement of pH to 5.20 after 96 hours

non-acidified solution, M_w remained unchanged, and the G(S) curve underwent no displacement. Thus, in spite of the span of incubation, crude DNase II retained a significant part of its activity, appearing only in favourable pH conditions.

Due to the complexity of the crude DNase II system, it seems likely that it is responsible for the pH fluctuations observed during DNA degradation. Proteolysis should be an important factor of pH increase, as judged from the fact that repeated treatments of crude DNase II with 0.2 % saponin solutions resulted in a deproteinization of the system and in a marked decrease of pH variations. After 6 such treatments the pH of crude DNase II incubated for 72 hours remained constant and the enzyme retained most of its activity (Fig. 3). Moreover, the hydrolysis products of the components of crude DNase II seemed to undergo

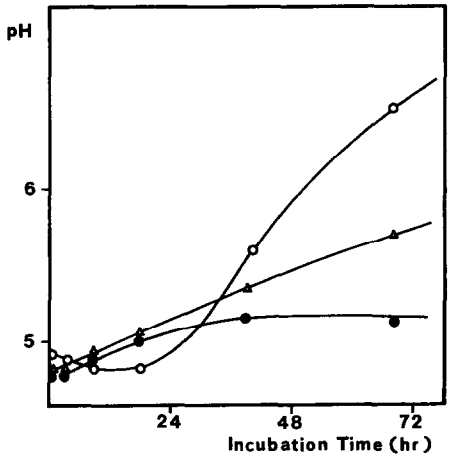

Figure 3 : Influence of the number of treatments of crude DNase II with 0.2 % saponin, on the pH variation during incubation
●——● 2 treatments ; Δ——Δ 4 treatments ;
o——o 6 treatments .

degradative oxidations, for when incubations were performed in the strict absence of O_2, with degassed reactants, no pH increase occured, whereas M_w decreased faster, reaching values below 5×10^5 daltons. General inhibitors of enzyme reactions, as EDTA, PCMB, AIA, or cysteine, added at a 10^{-4} M concentration, prevented pH variations and increased the nucleolysis rate.

REFERENCES

(1) G. BERNARDI, M. CHAMPAGNE and Ch. SADRON, Biochim. Biophys. Acta 49 (1961) 1
(2) G. BERNARDI and Ch. SADRON, Biochemistry 3 (1964) 1511
(3) R.S. WELSH, Proc. Natl. Acad. Sci. U.S. 48 (1962) 887
(4) R. LITZLER, Thesis Strasbourg (1967)
(5) D. PETTIJOHN and T. KAMYIA, J. Mol. Biol. 29 (1967) 275
(6) H. SEKINE, E. NAKANO and K. SAKAGUCHI, Biochim. Biophys. Acta 174 (1969) 202
(7) E.R.M. KAY, N.S. SIMONS and A.L. DOUNCE, J. Am. Chem. Soc. 74 (1952) 1724
(8) J. MARMUR, J. Mol. Biol. 3 (1961) 208
(9) V.N. SCHUMAKER and H.K. SCHACHMAN, Biochim. Biophys. Acta, 23 (1957) 628
(10) F.W. STUDIER, J. Mol. Biol. 11 (1965) 373
(11) D. FROELICH, C. STRAZIELLE, G. BERNARDI and H. BENOIT, Biophys. J. 3 (1963) 115
(12) G. BERNARDI, Adv. Enzymol. 31 (1968) 1