Eur. J. Biochem. *40*, 149–153 (1973)

The Specificity of *Escherichia coli* Endonuclease I

Stanislav D. Ehrlich, Umberto Bertazzoni, and Giorgio Bernardi

Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire, Paris

(Received July, 2 1973)

Degradation of different DNA's by *E. coli* Endonuclease I was studied. Hyperchromicity and acid solubility of the digest as well as the average chain length ($\bar{P}_n$) of oligonucleotides released by the enzyme were followed during degradation; relationships between these parameters were established.

The specificity of the *E. coli* Endonuclease I was determined by analysing 3′-terminal, 5′-terminal and 5′-penultimate nucleotide of oligonucleotides formed by the enzyme from calf thymus DNA. The compositions found differ from those expected on the basis of random breakage of DNA; this indicates that *E. coli* Endonuclease I recognizes sequences at least three nucleotides long.

The 3′-terminal nucleotide composition was determined over a wide range of $\bar{P}_n$ (150-4) and was found to be invariant, indicating that the specificity of *E. coli* Endonuclease I does not change during digestion.

Compositions of 3′-terminal nucleotides released from different bacterial DNA's varied linearly with the DNA (dG + dC) contents; those released from the calf thymus DNA did not fit that relationship.

Escherichia coli endonuclease I is a well-known DNAase (see [1] for a review) localized in the periplasmic space [2] which preferentially attacks native DNA and whose mechanism of degradation is characterized by an initial kinetics similar to that found for acid DNAase [3, 4].

In the present work we have investigated: (a) the changes of hyperchromicity, acid solubility, average chain length and 3′-terminal nucleotide composition of the digest, during degradation of calf thymus DNA by endonuclease I; (b) the isostich pattern in the terminal phase of digestion and the 3′-terminal nucleotide composition of different isostich classes; (c) the termini[1] of oligonucleotides released from different DNAs. As for the pancreatic DNAase [3], we have found that: (a) endonuclease I recognizes sequences at least three nucleotides long; (b) the specificity of the enzymes does not vary during the course of DNA degradation; (c) a linear relationship is obtained between the relative amounts of 3′-terminals released from different bacterial DNAs and their (dG + dC) contents; a deviation from this linearity is obtained in the case of calf thymus DNA.

[1] With this term we designate, conventionally, the 3′-terminal nucleotide and the 5′-terminal and penultimate nucleotides.

Abbreviations. $\bar{P}_n$ average degree of polymerization, average size or average chain length of oligonucleotides.

Enzymes. Endonuclease I from *E. coli* (EC 3.1.4.–); acid deoxyribonuclease from porcine spleen (EC 3.1.4.6); acid exonuclease from porcine spleen (EC 3.1.4.1); deoxyribonuclease from bovine pancreas (EC 3.1.3.5); acid deoxyribonuclease from *Helix aspersa*, Müll. (EC 3.1.4.–).

EXPERIMENTAL PROCEDURE

DNAs and enzymes were preparations described in the preceding paper [5].

E. coli endonuclease I was a preparation from *E. coli* B obtained by C. Cordonnier and G. Bernardi according to a procedure to be published elsewhere.

Endonuclease I digestions were performed at 25 °C on native DNA using solutions having an $A_{260} = 8.0$, in 0.05 M Tris-HCl pH 7.6, 0.01 M $MgCl_2$. Absorbance at 260 nm was followed during digestion; at different times aliquots were withdrawn and the reaction stopped by shaking the samples with 0.1 to 0.5 volumes of chloroform–isoamyl alcohol mixture (24:1, v/v).

Average size of oligonucleotides (average chain length, $\bar{P}_n$), and composition of 3′-terminal, 5′-terminal and 5′-penultimate nucleotides were determined as previously described [6–9] on samples dialyzed against running water [10]. DEAE-cellulose-7 M urea chromatography was performed as suggested by Tomlinson and Tener [11].

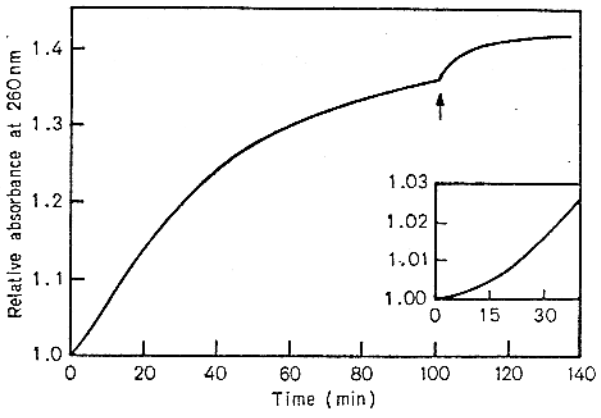

Fig. 1. *Hyperchromic shift of calf-thymus DNA during* E. coli *endonuclease I digestion.* The values refer to digestions of DNA solution, $A_{260} = 8.0$, in 0.05 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.6 at 25 °C with 2.2 U/ml (main figure) or 0.4 U/ml (insert) of endonuclease I. Arrow indicates further enzyme addition (2.2 U/ml)

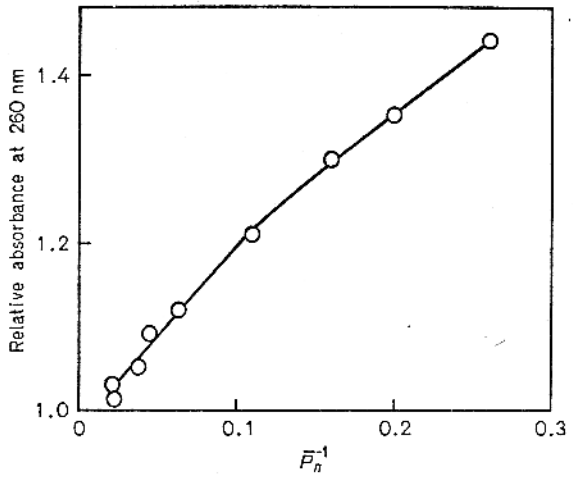

Fig. 2. *Plot of hyperchromic shift* versus *reciprocal average chain length of oligonucleotides.* Conditions of digestions and $\bar{P}_n^{-1}$ determinations were as described in Materials and Methods

Acid solubility of the digest was de ermined as described earlier [6].

RESULTS AND DISCUSSION

Kinetics of DNA Degradation

Fig. 1 shows the time course of the hyperchromic shift of calf thymus DNA during digestion by *E. coli* endonuclease I. As for the spleen [6], the snail [12] and the pancreatic [5] DNAase, the relationship is characterized by an initial lag time (see insert) followed by a linear increase of hyperchromicity during which melting of fragments takes place. The exhaustion of the preferred, double-stranded substrate is probably responsible for the progressive slowing down of the reaction [6].

A plot of the relative absorbance of endonuclease I hydrolysate against its reciprocal average size ($\bar{P}_n^{-1}$) gives a relationship (Fig. 2) differing from those obtained for spleen and snail DNAases by its biphasic pattern, and from that of pancreatic DNAase for lacking the first non-linear part. The part of the curve shown in Fig. 2 with $\bar{P}_n^{-1}$ values between 0.02 and 0.11 can be approximated by the equation:

$$\bar{P}_n^{-1} = 0.0044 \cdot HS + 0.0125 \qquad (1)$$

where HS is the hyperchromic shift at 260 nm expressed as a percentage. This equation can be used in order to prepare oligonucleotides of desired $\bar{P}_n$ simply by measuring the increase of absorbance during digestion of calf thymus DNA.

The increment of relative absorbance per broken bond, which is proportional to the constant factor of Eqn (1), is found to be very close to those obtained with spleen DNAase [6], snail DNAase [12] and pancreatic DNAase [5].

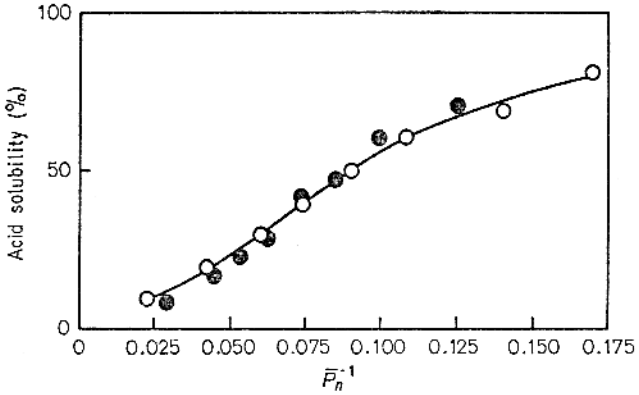

Fig. 3. *A plot of the relative acid solubility of* E. coli *endonuclease I digest against its reciprocal size.* The results correspond to the digestion of 28 ml calf thymus DNA solution, $A_{260} = 8.2$ in 0.05 M Tris-HCl pH 7.6, 0.01 M $MgCl_2$ at 25 °C with 3 U/ml endonuclease I. $\bar{P}_n$ values were calculated from the hyperchromic shift determinations using the relationship of Fig. 2. Filled symbols correspond to the pancreatic DNAase data [3] shown here for the purpose of comparison

Fig. 3 shows a plot of the relative acid solubility of *E. coli* endonuclease I digest against its reciprocal average size. As for the pancreatic DNAase, whose results are given for comparison, the relationship is slightly sigmoidal, while that of the spleen DNAase is linear. The acid-solubility of the spleen DNAase digest is linear during the early phase ($\bar{P}_n^{-1} < 0.08$) and increases exponentially to completeness at a $\bar{P}_n^{-1} = 0.1$, while that of the endonuclease I slows down and is close to 75% at a $\bar{P}_n^{-1} = 0.125$.

The Isostich Pattern in the Terminal Phase of Digestion

The distribution of the oligonucleotides at the late terminal phase of degradation of calf thymus DNA

Table 1. *Distribution of the oligonucleotides at the terminal phase of degradation of calf-thymus DNA as obtained by DEAE-cellulose fractionation*
See legend of Fig.4 for details. Numbers in brackets after isostichs are the percentage of each class. Terminal nucleotides were not determined for classes 1, 2, 7 and 8

3′-Terminal nucleotide	Isostich class							
	1 (0%)	2 (9%)	3 (19%)	4 (26%)	5 (29%)	6 (13%)	7 (3%)	8 (1%)
			%					
dT			45	41	35	29		
dG			8	11	11	12		
dA			32	32	37	40		
dC			14	16	17	18		

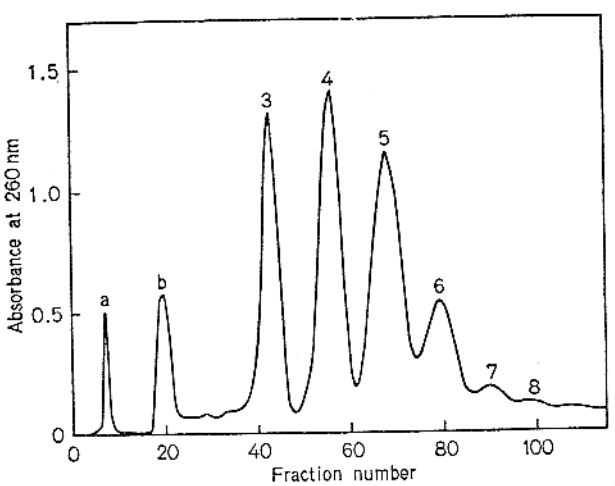

Fig.4. *DEAE-cellulose-7 M urea chromatography of* E. coli *endonuclease I digest.* 2 ml calf thymus DNA was digested under conditions of Fig.1 to a hyperchromic shift of 44% and adjusted to pH 5.5 by adding 1 M acetic acid. Digest was dephosphorylated with 0.1 U/ml acid phosphomonoesterase B at 25 °C for 16 h and loaded on a DEAE-cellulose column (0.5×15 cm). The column was washed using a flow-rate of 6 ml/h first with water (10 ml) and then with 7 M urea (10 ml). A linear gradient (0.04 M ammonium acetate in 7 M urea, total volume 100 ml) was applied in order to elute oligonucleotides. Fractions of 1 ml were collected and their absorbance at 260 nm measured. The numbers indicate the size of isostichs. Dinucleoside-monophosphates were partially eluted during loading (peak a), and partially with the beginning of gradient (peak b). No nucleosides were found when peak a was analyzed on the Aminex A6 column

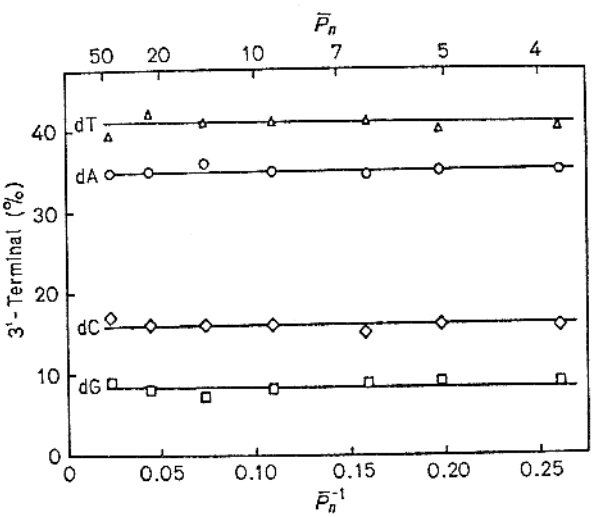

Fig.5. *Analysis of 3′-terminals in isostichs of calf-thymus DNA obtained after* E. coli *endonuclease I digestion.* Conditions of digestion and separation of isostichs are described in the legend of Fig.4

is given in Table 1. The chromatographic pattern of the dephosphorylated hydrolysate on DEAE-cellulose-urea columns is shown in Fig.4. They are characterized by: (a) the absence of mononucleotides; (b) a series of peaks formed by di-, tri-, tetra-, penta-, exanucleotides, the tetra and the penta-nucleotide peaks being predominant; (c) a very small fraction formed by poorly resolved peaks and the absence of unresolved material.

Composition of Termini

Fig.5 shows the relative amounts of 3′-terminal nucleotides released by the endonuclease I from calf thymus DNA plotted against the reciprocal average size of the digest. The compositions are invariant at least at the size levels studied ($\bar{P}_n$ comprised between 50 and 4). 3′-Terminals were also determined at a much higher size level, ($\bar{P}_n \approx 150$) by terminal labeling of oligonucleotides as previously described [9]; their composition (39% thymidine, 10% deoxyguanosine, 36% deoxyadenosine and 15% deoxycytidine) was in excellent agreement with the values indicated in the Fig.5. These results show that the specificity of the *E. coli* endonuclease I does not vary during digestion. Complete digestion of calf thymus DNA at 37 °C did not change the 3′-terminal composition (40% thymidine, 10% deoxyguanosine, 34% deoxy-

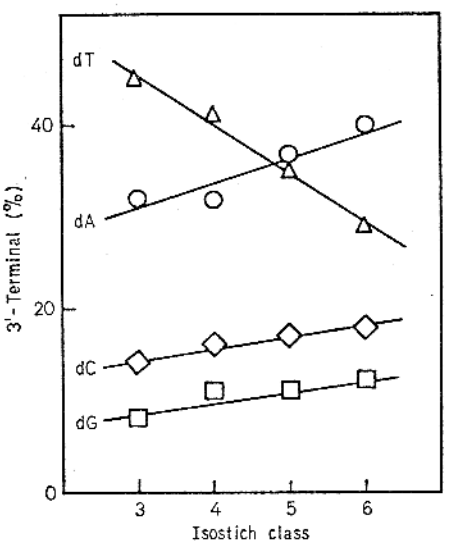

Fig. 6. *3'-Terminal nucleotides obtained at different degradation levels of calf-thymus DNA by* E. coli *endonuclease I*

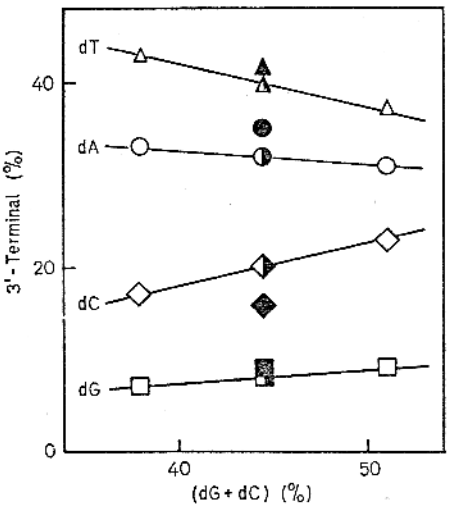

Fig. 7. *Plot of the percentage of 3'-terminal nucleotide as a function of DNA (dG + dC) contents.* Open, half-filled and filled symbols correspond to 3'-terminals released from bacterial DNAs, mixture of bacterial DNAs and calf thymus DNA, respectively

adenosine, 15% deoxycytidine) while degradation of denatured calf thymus DNA at 25 °C yielded a higher value for deoxyguanosine (15%) and a slight decrease for thymidine (38%) and deoxyadenosine (32%). The 3'-terminals of different classes of isostichs were also analyzed; the compositions are similar to those of the unfractionated hydrolysate (Table 1) but showed a tendency to increase with the class number for deoxyadenosine, deoxycytidine and deoxyguanosine and to a rather sharp decrease for thymidine (Fig. 6).

Table 2 shows compositions of 3'- and 5'-terminal and 5'-penultimate nucleotides released from calf thymus DNA by endonuclease I. The results obtained differ from (a) the base composition of the DNA (dG + dC = 44%); (b) the compositions calculated from the neighbor frequences [13]. These results indicate that the *E. coli* endonuclease I recognizes sequences at least three nucleotides long. However the average composition of termini split by the endonuclease I is different from that found for the pancreatic DNAase, showing that the two enzymes recognize a different set of nucleotide sequences. This is particularly evident for the 5'-terminal position: thymidine and deoxyguanosine are predominant in the pancreatic and endonuclease I digests, respectively, While the amount of 3'-terminal deoxycytidine is the same in calf thymus DNA and in the bacterial DNA mixture in the case of the pancreatic DNAase, a substantial differences is seen for the endonuclease I with a 4% increase in the bacterial DNA mixture. This might reflect the shortage of the dCpdG sequence in eukaryotes [13] and suggests that the *E. coli* DNAase recognizes, among other sequences, the dCpdG doublet. Table 3 shows

Table 2. *Average composition of termini released by* E. coli *endonuclease I from calf-thymus DNA*
Values in parentheses indicate the composition of each terminus as calculated from its nearest neighbor(s)

Nucleotide	3'-Terminal		5'-Terminal			5'-Penultimate	
	Expt	Calcd	Calcd	Expt	Calcd	Calcd	Expt
	%						
dT	41	(31)	(29)	24	(30)	(29)	28
dG	8	(20)	(22)	35	(20)	(20)	29
dA	35	(30)	(28)	17	(30)	(30)	29
dC	16	(19)	(20)	23	(20)	(21)	14

Table 3. *3'-Terminals released by* E. coli *endonuclease I*

3'-Terminal nucleotide	*H. influenzae* (dG + dC = 38%)	*Mixture* (dG + dC = 44.5%)	*E. coli* (dG + dC = 51%)
	%		
dT	43	40	37
dG	7	8	9
dA	33	32	31
dC	17	20	23

the 3'-terminals released by the endonuclease I from two bacterial DNAs (*Haemophilus influenzae* and *E. coli*) and the 1:1 mixture of the two. A linear relationship is found to hold between the percentage of terminals released and the (dG + dC) contents of the bacterial DNAs as shown in Fig. 7. The com-

position of 3′-terminals formed by digestion of calf thymus DNA deviate from that relationship (Fig. 7). The linear relationship for bacterial DNA is similar to that found for all DNAases studied so far [5, 6, 12] and the deviation from this linearity in the case of calf thymus DNA is expected for the same reasons discussed for the pancreatic DNAase (see preceding paper). The results obtained extend to endonuclease I the conclusion that the DNAase recognize nucleotide sequences and can be used to study nucleotide sequences in DNA.

U. B. is a Euratom scientific agent. This publication is contribution 897 of the Euratom Biology Division. The work was supported, in part, by a grant from the *Délégation Générale à la Recherche Scientifique et Technique* (Paris, France).

REFERENCES

1. Lehman, I. R. (1971) in *The Enzymes*, 3rd ed., (P. D. Boyer, ed.), vol. 4, p. 251, Academic Press, New York.
2. Cordonnier, C. & Bernardi, G. (1965) *Biochem. Biophys. Res. Commun. 20*, 555–559.
3. Bernardi, G. & Cordonnier, C. (1965) *J. Mol. Biol. 11*, 141–143.
4. Studier, F. W. (1965) *J. Mol. Biol. 11*, 373–390.
5. Ehrlich, S. D., Bertazzoni, U. & Bernardi, G. (1973) *Eur. J. Biochem. 40*, 133–137.
6. Soave, C., Thiery, J. P., Ehrlich, S. D. & Bernardi, G. (1973) *Eur. J. Biochem. 38*, 423–433.
7. Thiery, J. P., Ehrlich, S. D., Devillers-Thiery, A. & Bernardi, G. (1973) *Eur. J. Biochem. 38*, 434–442.
8. Bernardi, G., Ehrlich, S. D. & Thiery, J. P. (1973) *Methods Enzymol.*, in press.
9. Bertazzoni, U., Ehrlich, S. D. & Bernardi, G. (1973) *Biochim. Biophys. Acta, 312*, 192–201.
10. Devillers-Thiery, A., Ehrlich, S. D. & Bernardi, G. (1973) *Eur. J. Biochem. 38*, 416–422.
11. Tomlinson, R. V. & Tener, G. M. (1963) *Biochemistry, 2*, 697–702.
12. Laval, J., Thiery, J. P., Ehrlich, S. D., Paoletti, C. & Bernardi, G. (1973) *Eur. J. Biochem. 40*, 133–137.
13. Swartz, M. N., Trautner, T. A. & Kornberg, A. (1962) *J. Biol. Chem. 237*, 1961–1967.

S. D. Ehrlich's present address: Department of Genetics, Stanford University School of Medicine, Stanford California, U.S.A. 94305

U. Bertazzoni and G. Bernardi, Institut de Biologie Moléculaire, Université de Paris VII, 2 Place Jussieu F-75005 Paris, France