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Mechanism of Degradation of DNA by Endonuclease I from *Escherichia coli*

Work from this laboratory has shown that acid DNase, an enzyme recently obtained as a homogeneous protein from hog spleen (Bernardi, Griffé & Appella, 1963; Bernardi & Griffé, 1964), is able to degrade DNA according to both "double hit" and "single hit" kinetics (Bernardi & Sadron, 1961,1964; MacHattie, Bernardi & Thomas, 1963). While the former mechanism is associated with random breaks on one strand or the other and is similar to that already known for pancreatic DNase (Thomas, 1956; Schumaker, Richards & Schachman, 1956), the latter implies, if DNA molecules are continuous double-stranded structures, the simultaneous breakage of the two strands at the same level.

A comparative study of acid DNase preparations from different tissues and sources (Cordonnier & Bernardi, 1964), while confirming the very widespread and possibly universal presence of an acid DNase activity in the cells of multicellular organisms, has shown that this activity is carried by protein molecules having the same chromatographic, ultracentrifugal and enzymological properties.

Since no enzyme with properties similar to those of acid DNase has been described so far in bacteria, and yet the distribution and the peculiar mechanism of degradation found for acid DNase suggest a general, although still obscure, role for this enzyme, an investigation was carried out to see whether some known bacterial DNase was able to degrade DNA like acid DNase. *Escherichia coli* endonuclease I (Lehman, 1963) was chosen for this work because it resembles acid DNase in several respects: both enzymes have a particulate nature, preferentially attack native DNA yielding large-sized oligonucleotides, have an alkaline isoelectric point and are competitively inhibited by S-RNA and poly A–poly U (Lehman, Roussos & Pratt, 1962*a*,*b*; Bernardi & Griffé, 1964; Bernardi, 1964). There is no doubt, however, that they are different proteins; in contrast to acid DNase, the bacterial enzyme is a 5′-phosphomonoester former, requires Mg^{2+} and has an alkaline pH optimum. The *E. coli* DNase preparation used in this work was obtained by Dr H. E. Schaller according to Lehman *et al.* (1962*a*). Crystalline pancreatic DNase (lot 932) was purchased from Worthington, Freehold, N.J., U.S.A. Calf thymus DNA and hog spleen acid DNase were prepared as previously described (Bernardi & Sadron, 1964; Bernardi & Griffé, 1964). Enzymic degradations were followed by light scattering or by viscometry, using a four-bulb viscometer (Eigner, 1960). The experimental conditions and the treatment of the kinetic data have been described in detail elsewhere (Bernardi & Sadron, 1964).

Typical kinetic data obtained with the three different enzyme preparations mentioned above are presented in Table 1. An example of DNA degradation by *E. coli* DNase is shown in Fig. 1. These results show that the bacterial enzyme initially degrades DNA according to "single hit" kinetics, like acid DNase, whereas the expected "double hit" kinetics is found with pancreatic DNase. Preliminary results obtained at longer digestion times indicate that *E. coli* DNase is able to cause in addition random breaks on one or the other of the two strands, a phenomenon already noticed with acid DNase (Bernardi & Sadron, 1961,1964).

TABLE 1

Enzymic degradation of DNA

Enzyme	n[a] (light-scattering)	n[a] (viscometry)
Acid DNase	1·0 ± 0·1[b]	1·0 ± 0·1
Pancreatic DNase	1·7 — 2·0[b]	1·5 ± 0·1[c,d]
E. coli DNase	1·0 ± 0·1[e]	1·0 ± 0·1[d]

[a] The values of n reported in this Table were obtained in a large number of experiments. n is the apparent number of strands, as obtained from a plot of log $(1 - R)/R$ *versus* log t. R is equal to M_t/M_o or to η_t/η_o, where M_t, η_t, M_o, η_o are the molecular weights or the reduced viscosities (at a shear gradient of about 45 sec^{-1}) at time t and time 0 of digestion, respectively.

[b] Data of Bernardi & Sadron (1964).

[c] A low n value (1·5 as against the theoretical value of 2·0), as determined by viscometry, was reported by Schumaker *et al.* (1956).

[d] The solvent was 0·1 M-acetate buffer, pH 5·0, + 0·02 M-Mg Cl_2.

[e] The solvent was 0·066 M-tris buffer, pH 7·5, + 0·006 M-Mg Cl_2.

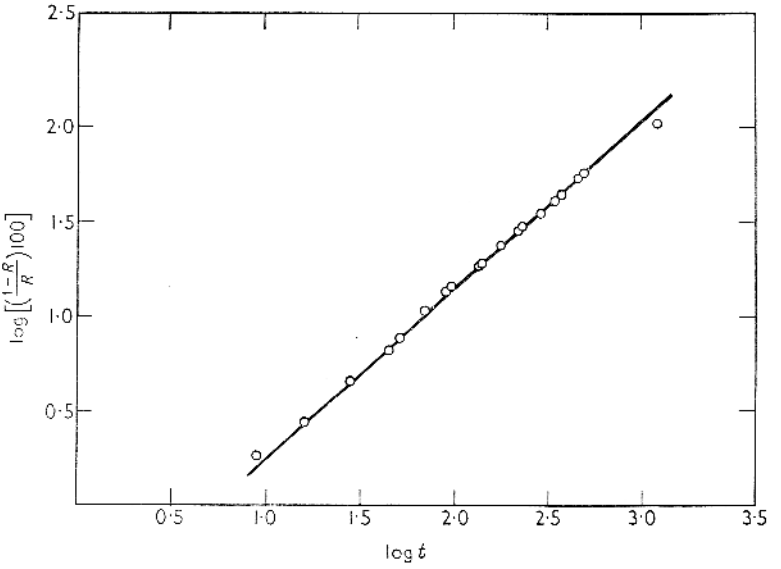

FIG. 1. Degradation of calf thymus DNA by *E. coli* DNase, as followed by viscometry. Only one bulb of the viscometer was used (shear gradient of about 45 sec^{-1}). R is defined in footnote (a) to Table 1; t is the digestion time; the slope of the straight line is equal to n, the apparent number of strands.

These findings are interpreted as indicating that *E. coli* DNase, like acid DNase, can simultaneously split both DNA strands at the same level (see Bernardi & Sadron, 1964, for a discussion). The discovery of a "single hit" DNA degradation by a bacterial enzyme suggests that the acid DNase of multicellular organisms has a counterpart in bacteria.

Nothing can be said at the present time about the physiological role of this class of enzymes; however, their possible implication in recombination and, more generally, in breakage and re-union processes must be seriously considered and investigated.

Identical conclusions were reached independently by Dr William Studier, working with λ phage DNA and using zone sedimentation to follow the enzymic degradation (private communication).

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