LOCALIZATION OF E. COLI ENDONUCLEASE I

by

Claude CORDONNIER and Giorgio BERNARDI
Centre de Recherches sur les Macromolécules,
Strasbourg, France

Received July 14, 1965

Work from this laboratory has shown that acid DNAase from several sources (Bernardi and Sadron, 1961, 1964) and E. Coli endonuclease I (Bernardi and Cordonnier, 1965) initially degrade DNA according to a "single hit" kinetics, a mechanism implying the simultaneous breakage of the two DNA strands at the same level. While the acid DNAase activity of multicellular organisms, which is carried by protein molecules having the same chromatographic, ultracentrifugal and enzymological properties (Cordonnier and Bernardi, 1965) has been found in the lysosomes (De Duve, Wattiaux and Baudhuin, 1962), the DNAase activity of E.Coli has been reported as associated with the ribosomes (Elson, 1959 ; Tal and Elson, 1963) or in the non-sedimentable supernatant fraction of cell-free extracts (Shortman and Lehman, 1964). The recent work of Malamy and Horecker (1961, 1965) and Neu and Heppel (1964, a,b,c) has provided good evidence for the surface localization in E. Coli of several hydrolase activities, many of which are found in the lysosomes of multicellular organisms, and the presence of an RNA-inhibitable DNAase has been reported among the surface enzymes (R.J. Hilmoe, unpublished work, quoted by Neu and Heppel, 1964 a,b,c).

An investigation on the intracellular localization of E. Coli endonuclease I was considered of interest both as an approach to an understanding of its physiological role and in setting up a new isolation procedure for this enzyme. This was done by comparing several properties

of the DNAase released by lysozyme-EDTA treatment of E. Coli cells and endonuclease I.

E. Coli B/r cells were grown on a synthetic medium and harvested by centrifugation in a refrigerated Sharples centrifuge in the late exponential phase. A cell suspension was prepared and treated with lysozyme-EDTA according to Neu and Heppel (1964a). Several enzymatic

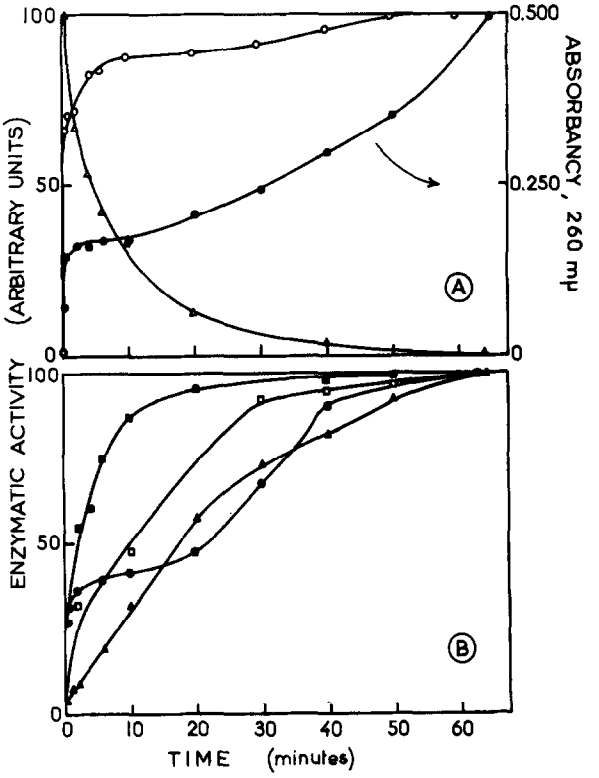

Figure 1

Time course of the liberation of enzymatic activities from EDTA-lysozyme treated E. Coli B/r cells. The enzymatic activities measured after about one hour incubation were taken as 100. After 15 hours incubation activities were only about 20% higher than those determined after one hour.

A. o optical density at 600 mμ of cells after the osmotic shock ; ● optical density at 260 mμ of protoplast supernatant ; Δ DNAase activity of spheroplasts.

B. ■ basic phosphatase ; □ acid phosphatase ; ▲ DNAase ; ● acid RNAase.

activities were assayed, according to methods described elsewhere (Bernardi and Griffé, 1964), in the supernatants obtained by centrifuging the cell suspension at different incubation time intervals. The protoplast formation (as measured by lysability), the ultraviolet absorption at 260 mμ of the supernatants and the DNAase activity of lysed protoplasts were also determined. The results obtained are shown in fig. 1. Acid and basic phosphatases, and acid RNAase were immediately released, as already shown by Neu and Heppel (1964c). The DNAase activity was apparently released more slowly, and its increase in the protoplast supernatant was paralleled by its decrease in the protoplast lysate. No DNAase activity was liberated from cell wall preparations upon lysozyme-EDTA treatment.

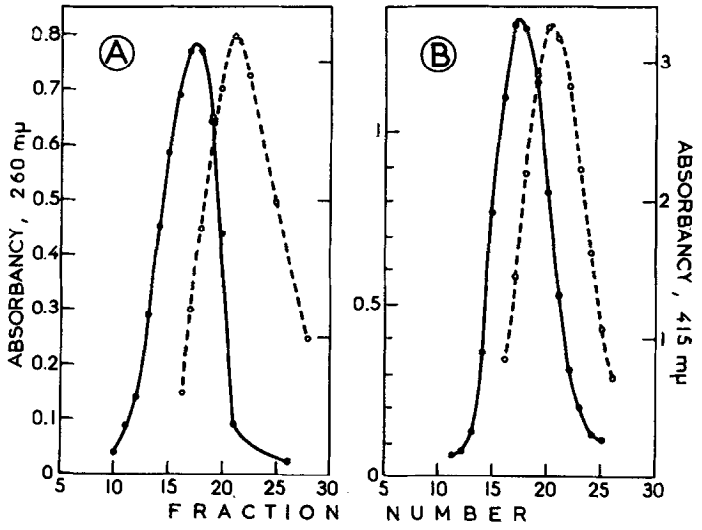

Figure 2

Sucrose-gradient centrifugation of E. Coli endonuclease I (B) and E. Coli DNAase released by EDTA-lysozyme treatment of E. Coli B/r cells, (A). A total of 28 and 26.5 fractions was collected, respectively. Circles indicate the absorption at 415 mμ of cytochrome c (right-hand ordinate) ; points indicate the DNAase activity (left-hand ordinate). Bottom of the cell to the left. A linear molarity gradient was obtained using 5% and 20% sucrose solutions in 0.066 M Tris buffer + 0.0066 M $MgCl_2$ pH 7.5. Centrifugation was carried out for 16 hours at 4° at 38,000 rpm using a SW-39 rotor and a Spinco Model L ultracentrifuge.

The lysozyme-EDTA released DNAase, as obtained in the spheroplast supernatant, was then compared with an endonuclease I preparation obtained according to Lehman, Roussos and Pratt (1962). a) Sucrose gradient centrifugation : both enzymatic activities showed a sedimentation coefficient equal to 2.5-2.6, using cytochrome c ($s^{\circ}_{20,w}$ = 1.7 S) as a reference protein (fig. 2). b) Chromatographic properties : both enzymes were not retained by DEAE-Sephadex columns equilibrated with 0.05 M phosphate buffer pH 6.8 ; they were eluted at a molarity of 0.25 M by a linear gradient (0.05 to 0.5 M) of phosphate buffer pH. 6.8 from both hydroxyapatite and CM-Sephadex columns. c) RNA inhibition : both enzymes were competitively inhibited by yeast s-RNA ; the surface enzyme showed a higher degree of inhibition than the purified endonuclease I preparation, a phenomenon which requires further work to be explained.

The results obtained very strongly suggest that the lysozyme-EDTA released DNAase is to be identified with endonuclease I. This surface localization of endonuclease I appears to be an interesting finding for an enzyme which might be somehow related to recombination mechanisms (Shortman and Lehman, 1964 ; Bernardi and Cordonnier, 1965), particularly in view of the location at the bacterial cell membrane of the growing point of DNA (Jacob, Brenner and Cuzin, 1963) and of DNA polymerase (Ganesan and Lederberg, 1965).

Acknowledgements

The authors thank Prof. C. SADRON for his interest in the present work.

References

Bernardi G., Cordonnier C., J.Mol.Biol. 11, 141 (1965)
Bernardi G., Griffé M., Biochemistry, 3, 1419 (1964)
Bernardi G., Sadron, C., Nature 191, 809 (1961)

Bernardi G., Sadron C., Biochemistry 3, 1411 (1964)
Cordonnier C., Bernardi G., Biochem. J. 94, 12 P (1965)
de Duve C., Wattiaux R., Baudhuin P., Advan.Enzymol. 24 291 (1962)
Elson D., Biochem.Biophys. Acta, 36, 372 (1959)
Ganesan A.T., Lederberg J., Biochem.Biophys.Res.Comm. 18, 824 (1965)
Jacob F., Brenner S., Cuzin F., Cold Spring Harbor Symp. Quant.Biol. 28, 329 (1963)
Lehman I.R., Roussos G.C., Pratt E.A., J.Biol.Chem. 237 819 (1962)
Malamy M.H., Horecker B.L., Biochem.Biophys.Res.Comm. 5, 104 (1961)
Malamy M.H., Horecker B.L., Biochemistry 3, 1889 (1964)
Neu H.C., Heppel L.A., Biochem.Biophys. Res.Comm. 14, 109 (1964a)
Neu H.C., Heppel L.A., Biochem.Biophys.Res.Comm. 17, 215 (1964b)
Neu H.C., Heppel L.A., J.Biol.Chem. 239, 3893 (1964c)
Shortman K., Lehman I.R., J.Biol.Chem. 239, 2964 (1964)
Tal M., Elson D., Biochem.Biophys. Acta 72, 439 (1963)