DETECTION OF RESTRICTION ENDONUCLEASES WITH A *lac* REPRESSOR–*lac* OPERATOR FILTER BINDING ASSAY

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1. Introduction

The use of restriction enzymes as experimental tools has progressed rapidly within the last several years [1]. The purification of these enzymes, however, often poses a problem: since acid-soluble material is normally not released a convenient assay for enzyme activity does not exist. Thus, gel electrophoresis or viscometry of digested DNA has often been employed to detect restriction enzymes during purification. These methods are, however, time comsuming and involve large quantities of DNA and/or expensive equipment. We report here a test for restriction enzymes that avoids these complications; the test is inexpensive, rapid and can be done with very small quantities of DNA (0.02 μg per test is normally sufficient).

2. Materials and methods

2.1. *Preparation of* lac *repressor*

E. coli lac repressor was prepared by published procedures [2] from strain BMH 606 (provided by B. Müller-Hill).

2.2. *Assay of* lac *repressor activity*

λ *plac* 5 was prepared from strain BMH 782 (obtained from B. Müller-Hill; originally obtained from J. Shapiro). The preparation of ^{32}P- λ*plac* DNA and the assay of *lac* repressor binding was by standard procedures [3].

2.3. *Assay of Eco RI restriction endonuclease*

Eco RI restriction endonuclease was prepared by DEAE-cellulose chromatography as described in the legend to fig.1. The assay procedure is as follows: 100 μl of ^{32}P-λ*plac* DNA (approximately 0.5 μg of DNA at 16×10^3 cpm/μg) were mixed with 10 μl of the indicated column fraction (see fig.1) and 1.0 ml of BB buffer (0.01 M Tris–HCl (pH 7.5), 0.01 M $Mg(OAc)_2$, 5×10^{-3} M KCl, 0.01 M mercaptoethanol 10^{-4} M EDTA, 50 μg/ml bovine serum albumin). After 2 h at 37°C 100 μl of a 1 mg/ml calf thymus DNA solution was added followed by 10 μl of a *lac* repressor solution (this amount of *lac* repressor is saturating). The mixtures were incubated 15 min at room temperature. Two samples of 450 μl were then passed slowly through nitrocellulose filters and the filters washed once with 300 μl of WB buffer (BB buffer without bovine serum albumin). The filters were then dissolved in Bray's solution [4] and counted in a liquid scintillation counter.

3. Results and discussion

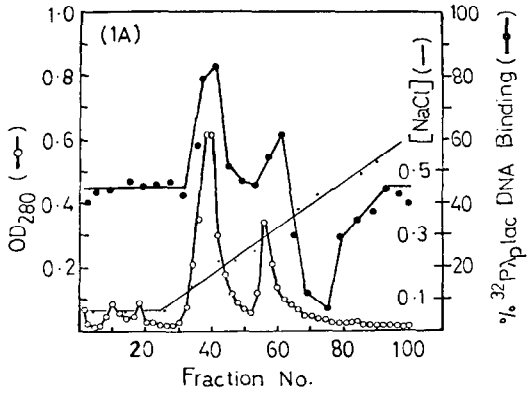
The *lac* repressor–*lac* operator test relies upon the fact that *E. coli lac* repressor will pick out and bind *lac* operator-containing DNA in a mixture of DNA molecules. The *lac* repressor–*lac* operator complexes are trapped on nitrocellulose filters while non-complexed DNA is not [3]. When using ^{32}P-λ*plac* DNA the number of cpm bound/filter depends upon the size of the DNA molecule containing the operator sequence, thus allowing the detection of endonucleases that do not digest DNA to acid-soluble products. The only prerequisite is that the enzyme make at least one cleavage in the λ*plac* DNA (not too close to one end if there is only a single cleavage).

Fig.1 demonstrates the method. A freeze–thawed

extract of *E. coli* (RI) was prepared and subjected to DEAE-cellulose chromatography. Aliquots of selected fractions were then incubated with ^{32}P-λ*plac* DNA and, following the addition of and incubation with *lac* repressor, passed through nitrocellulose filters (see Materials and methods and the legend to fig.1A). The large reduction in bound counts in fractions 65–80 indicates the presence of nuclease activity (alternatives, such as an anti-repressor activity, have not been encountered). These fractions were then shown to contain Eco RI enzyme by standard gel electrophoresis procedures (fig.1B).

Fig.1 also demonstrates a pitfall in the method. Between fractions 35–45 binding activity increases (fig.1A) even though a non-specific nuclease is present (fig.1B) Complete disruption of *E. coli* cells (ie. French press, sonication) results, in fact, in the release of such quantities of DNA-binding proteins as to make the filter binding test unusable. Complete disruption should, however, not normally be necessary since most or all *E. coli* restriction enzymes are released by osmotic shock ([5], and J. E. Davies, personal communication).

Hemophilus strains, as opposed to *E. coli,* do not appear to release large quantities of DNA-binding proteins. After a preliminary Sephadex chromatography step [6] the filter binding test can, therefore, be used with completely disrupted *Hemophilus* cells (H-J. Kreutzfeldt, unpublished experiments). We have not examined species other than *Escherichia* and *Hemophilus.* However, even in the event that large quantities of DNA-binding proteins are released, the filter binding assay should prove useful in the final stages of purification once these proteins have been removed.



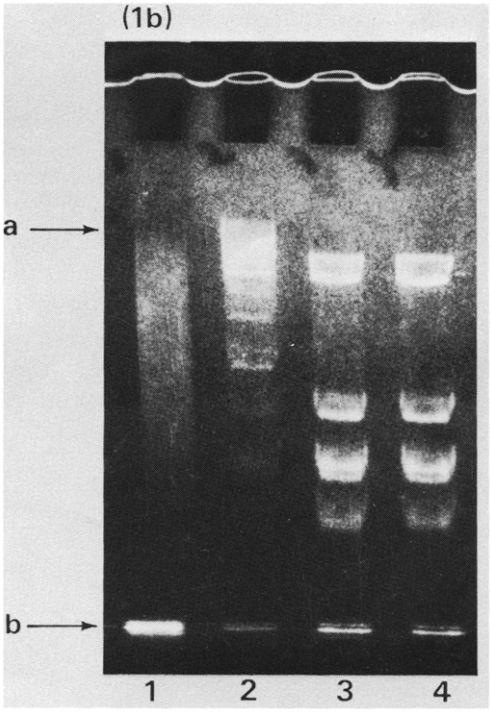


Fig.1(A) DEAE-cellulose chromatography of Eco RI restriction endonuclease. Eco RI restriction endonuclease was prepared by a modification of published procedures [7]. 46 g of frozen *E. coli* (RI) (grown by Merck, Darmstadt) were thawed in 50 ml PEM buffer (10^{-2} M potassium phosphate (pH 7.0), 10^{-2} M mercaptoethanol, 10^{-3} M EDTA). After centrifugation the supernatant was treated successively with streptomycin sulfate and ammonium sulfate [7] and dialyzed against PEM buffer containing 0.05 M NaCl. The dialyzed solution was loaded on a 1.2 × 22 cm DEAE cellulose column in PEM buffer containing 0.05 M NaCl. After washing the column with 100 ml of buffer the proteins were eluted with a linear gradient of 0.05–1.0 M NaCl in PEM buffer. Fractions of 4.6 ml were collected at 35 ml/h and monitored for OD_{280} and for Eco RI activity as described in Materials and methods. (B) Agarose gel electrophoresis of λDNA incubated with aliquots of fractions from the DEAE-cellulose column. Approximately 5 μg of λDNA was incubated for 2 h at 37°C in BB buffer with 20 μl of the following column fractions: slot 1, fraction 39; slot 2, fraction 56; slot 3, fraction 61; slot 4, fraction 72. The position of undegrated λDNA is shown by (a). The boundary between the agarose portion of the gel. (0.8% agarose) and the polyacrylamide portion (10% polyacrylamide) is shown by (b). Agarose gel electrophoresis was performed by a method developed in this laboratory [8].

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References

[1] Murray, K. and Old, R. (1974) Prog. in Nucleic Acid Res. and Mol. Biol. 14, 117–185.
[2] Müller-Hill, B., Beyreuther, K. and Gilbert, W. (1971) Methods in Enzymol. 21D, 483–487.
[3] Riggs, A., Suzuki, H. and Bourgeois, S. (1970) J. Mol. Biol. 48, 67–83.
[4] Bray, G. (1960) Anal. Biochem. 1, 297–305.
[5] Tanaka, T. and Weisblum, B. (1975) J. Bacteriol. 121, 354–362.
[6] Sharp, R., Sugden, G. and Sambrook, J. (1973) Biochemistry 12, 3055–3063.
[7] Yoshimuri, R. (1971) Ph. D. thesis, University of California, pp. 73.
[8] Schreier, P. (1974) Diplomarbeit, Universität zu Köln, pp. 42.