RAPID PURIFICATION OF A RESTRICTION ENDONUCLEASE FROM *Escherichia coli* RY13

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SUMMARY

A two step method for the purification of restriction endonuclease Eco RI was developed. The first step involved the purification of the enzyme on Cibacron Blue-F3GA-agarose column, followed by a hydroxyapatite column. The enzyme was homogeneous on SDS-PAGE and completely free from contaminating nucleases and phosphatases, and can be used for direct DNA hydrolysis.

INTRODUCTION

Restriction endonucleases play a major role in recombinant DNA technology. Various protocols have been developed for their purification, involving different chromatographic steps (Green et al., 1978). We have exploited the applicability of dye-ligand chromatography in the purification of Eco RI. The method is economical, effective and can be scaled up easily. The methodology has a potential for its extension to purify other restriction endonucleases.

MATERIALS AND METHODS

Cibacron Blue-F3G-A was covalently attached to 4% cross-linked agarose (Lowe, 1984). Bacteriophage λ DNA was isolated from *E. coli* λ CI 857 S7 according to the procedure of Rembhotkar and Khatri (1989). T4 DNA ligase was from New England Biolabs. Hydroxyapatite was from Bio-Rad Laboratories.

Growth of Cells

*Escherichia coli* RY13, from Prof. H.K. Das, Jawaharlal Nehru University, India, was grown at 37°C in 14 litres LB medium at 300 rev/min agitation and 11 litres/min aeration, to late log phase. The cells were harvested by using tangential flow filtration cassette system of Millipore. The yield of cells was 3-4 g/l (wet weight).

Measurement of Endonuclease Activity

The enzyme was assayed by using standard protocol (Sumegi et al., 1977). One unit of enzyme was

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defined as the amount required for complete digestion of 1 μg of λ DNA in 60 min at 37°C. The protein was determined by the method of Lowry with bovine serum albumin as standard.

Quality Control Tests

The purity of final Eco RI preparation with respect to ‘non- specific nucleases’ was evaluated by the ‘over-digestion’ and ‘cut-ligate-recut’ tests (Vlatakis et al., 1987).

(a) Over-digestion Tests

One μg of λ DNA was digested with 200 U of enzyme, at 37°C for 2, 4 and 6 h, to gain 400-, 800- and 1200- fold over-digestion products. These digestion products were analysed on agarose gel electrophoresis.

(b) Cut-ligate-recut Tests

In this test, 15 μg of λ DNA was digested completely, with 100 U of enzyme. These restriction fragments were isolated by conventional methods involving phenol-chloroform treatment. The fragments were then ligated with T4 DNA ligase. The ligation was verified on a mini-agarose gel. The ligated DNA was then recut by incubating with 50 U of Eco RI from the same lot.

Purification of Restriction Endonuclease

Escherichia coli RY13 (50 g, wet weight) were thawed, suspended in 200ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 5% glycerol, 400 mM NaCl, 7 mM MgCl₂, 10 mM 2-Mercaptoethanol), sonicated and centrifuged at 60000 g for 1 h. The supernatant was applied on Cibacron Blue-F3GA-agarose column (0.8 cm x 10 cm) and eluted with a linear gradient of 0.4 M to 1.5 M NaCl in buffer containing 50 mM Tris-HCl, pH 7.5, 5% glycerol, 5 mM EDTA, 10 mM 2-Mercaptoethanol. The enzyme activity was eluted between 0.7 M to 0.76 M NaCl. The pooled activity was further chromatographed on to a hydroxyapatite column (0.8 cm x 8 cm) and eluted with a linear gradient of 0.04 M to 0.4 M potassium phosphate buffer, pH 7.0. The active fractions were pooled and dialysed against storage buffer (300 mM NaCl, 5 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA, 5 mM 2-Mercaptoethanol, 0.15% Triton-X-100, 100 μg gelatin and 50% glycerol). The enzyme was stored at -20°C.

RESULTS AND DISCUSSION

The use of dye-ligand affinity chromatography as a purification tool for many enzymes including restriction endonucleases has been reported earlier (Lowe et al., 1986; Bouriotis et al., 1987).

Another group of workers (Baksi et al., 1978) reported a single step purification method for different restriction endonucleases. However, we found that a single step procedure was not sufficient for the preparation of a pure Eco RI for commercial use. A combination of Cibacron Blue-F3GA-agarose with hydroxyapatite was necessary. Thus, a cell free, dialysed, crude bacterial extract was applied on dye-bound agarose column. The unbound proteins were washed off with loading buffer, until no absorbance at 280 nm was evident in washings. The enzyme was then eluted with a gradient of NaCl. The endonuclease activity was pooled and subjected to hydroxyapatite column chromatography. The enzyme so obtained was homogeneous on SDS-PAGE. The whole chromatographic protocol is summarized in Table 1.