A NEW RESTRICTION ENDONUCLEASE *Bcn*I FROM *BACILLUS CENTROSPORUS* RFL 1

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Received 18 November 1981

1. Introduction

Type II restriction endonucleases have proved to be an indispensable tool in DNA cloning and sequencing studies. Over 200 individual enzymes with >60 different specificities have been already described (review [1]). Here, we describe the purification and the determination of cleavage specificity of a novel type II restriction endonuclease from *Bacillus centrosporus* RFL 1.

2. Experimental

Liquid bacterial culture medium (Penassay broth) was purchased from Difco. Ion exchangers were from Whatman, Sephadex G-100 from Pharmacia Fine Chemicals and heparin–agarose was prepared according to the method of [2]. All the other reagents including those for gel electrophoresis and DNA sequencing were of reagent grade unless otherwise specified.

Bacterial cultures were grown at 37°C with aeration to late logarithmic phase, then the cells (~2 g/l) were harvested by centrifugation at 10 000 × *g* for 10 min and stored frozen at −20°C.

Determination of enzyme activity was performed with phage λ DNA (CI 857) in a buffer containing 50 mM Tris–HCl (pH 7.5)–10 mM $MgCl_2$–25 mM NaCl–10 mM 2-mercaptoethanol. Aliquots (40 μl) of this mixture were incubated with 1–5 μl aliquots from column fractions (each portion also contained 1 or 2 μg DNA) for 1 h at 37°C. Products were analyzed by agarose gel electrophoresis. A unit of enzyme activity is defined as an amount of enzyme capable of completely digesting 1 μg λ DNA under conditions specified.

Enzyme isolation was by the following procedure: 20 g cells were suspended in 40 ml ice-cold 10 mM potassium phosphate (pH 7.5)–7 mM 2-mercaptoethanol–1 mM EDTA (herein buffer A) plus 0.2 M NaCl, disrupted by sonication and centrifuged at 40 000 × *g* for 1 h at 4°C. Clear supernatant was directly applied onto a heparin–agarose column (1.5 × 12 cm) pre-equilibrated in buffer A plus 0.2 M NaCl. Enzyme activity adsorbed on the column was eluted with a linear gradient of NaCl (0.2–0.7 M) in buffer A, containing 10% glycerol. Active fractions were pooled (0.47–0.54 M), diluted with an equal volume of buffer A and applied onto a phosphocellulose column (1 × 10 cm). Enzyme activity was eluted with a linear gradient of NaCl in buffer A (0.3–0.8 M) containing 10% glycerol. Active fractions were pooled (0.65–0.72 M) concentrated to a volume of several millilitres and applied onto a Sephadex G-100 column (2.5 × 90 cm) pre-equilibrated in buffer A, containing 10% glycerol and 0.5 M NaCl. Active fractions were pooled, concentrated by dialysis against buffer A, containing 0.2 M NaCl and 50% glycerol. The preparation was stored at −20°C for 3 months without apparent loss of activity. The above isolation procedure, usually yielded ~2×10^6 units (from 20 g cells) with a specific activity of 2×10^5 units/ml.

Determination of cleavage specificity was accomplished by 2 methods.

(i) pBR 322 plasmid DNA was cleaved with a novel enzyme and the sizes of the resulting fragments were determined by agarose gel electrophoresis (*Alu*I-fragments of the same DNA served as size markers). A set of lengths thus obtained was compared to several other sets constructed on the assumption that the cleavage site was a degenerate derivative of a *Sma*/*Xma* cleavage site (M. P. P., preliminary observations), CCCGGG;

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

(ii) A method described in [3] was used. A suitable short DNA fragment, containing a single site for a novel enzyme, was labelled at alternate strands and digested with a novel enzyme. The product was sized on a denaturing polyacrylamide gel, a chemical sequencing ladder serving as size markers.

3. Results and discussion

In the course of a systematic search for novel restriction enzyme activities we tested a strain of *Bacillus*, originally isolated as a contaminant of a regular culture of *Bacillus subtilis*. An unusual pattern of bands, obtained on agarose gel after cleavage of test DNAs was indicative of a novel activity present in the bacterium. We therefore undertook a purification of this activity and determined a cleavage specificity of a new restriction endonuclease. We also determined a systematic position of the isolated strain of *Bacillus*. Finally, we were successful in isolating an enzyme from *Bacillus centrosporus* RFL 1, which cleaves phage λ DNA at >50 sites, SV 40 DNA once, does not cleave φX 174 RF DNA and should be termed *Bcn*I according to the conventional nomenclature.

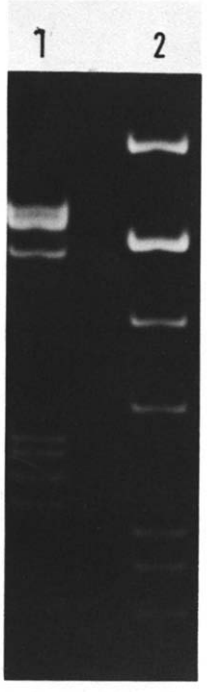

Fig.1. Agarose gel electrophoresis of pBR 322 DNA, cleaved with: (1) *Bcn*I; (2) *Alu*I.

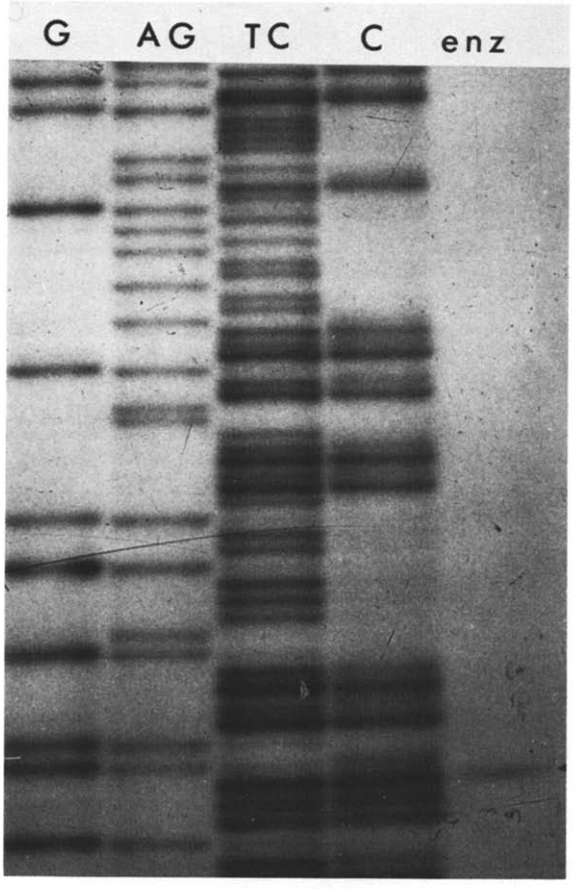

Fig.2. A sequencing gel (7% acrylamide–7 M urea–100 mM Tris–borate, pH 8.3) showing the exact sizing of the *Bcn*I cleavage product of an end-labelled DNA fragment against the chemical sequencing ladder of the same fragment (5).

An exact determination of cleavage specificity was performed by 2 methods (see section 2) which gave the same result: *Bcn*I cleaves a sequence 5′-CC$\overset{C\downarrow}{G}$GG-3′, as shown by the arrow. The gels, from which the above conclusion was inferred, are shown in fig.1,2.

The enzyme preparation obtained by the procedure described contains very little, if any, of the contaminating activities that could have interfered with DNA sequencing procedures. Though the activity tends to decrease after 3 months storage, an initial high specific activity that is routinely obtained, eliminates this problem.

While we were testing the stabilities of the enzyme preparation and of the source strain, a paper appeared [4], in which an enzyme with the same specificity was described for *Neisseria cinerea*.

Acknowledgements

The authors are grateful to Dr P. M. Rubtsov (Inst. Molecular Biology, USSR Academy of Sciences, Moscow) for expert testing *BcnI* in extensive DNA sequencing work, and Drs E. Lukanidin and G. Dolganov (respectively, Inst. Molecular Biology and Inst. Bioorganic Chemistry, USSR Academy of Sciences, Moscow) for generous gifts of SV 40 and ϕX 174 DNAs.

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