A new site-specific endonuclease, Scal, from Streptomyces caespitosus

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A new site-specific endonuclease has been isolated from Streptomyces caespitosus and named Scal. Based on analysis of sequences around the restriction sites in pBR322 and pBR325, the recognition sequence of Scal endonuclease was deduced to be a new hexanucleotide 5'-AGTACT-3'. The cleavage site was determined by comparing the Scal-cleaved product of a primer-extended M13mp18-SCA DNA, which contains an AGTACT sequence, with dideoxy chain terminator ladders of the same DNA. Scal was found to cleave the recognition sequence between the internal T and A, leaving flush ends to the cleaved fragments.

INTRODUCTION

A number of restriction endonucleases have been isolated from many species of procaryotes and their specificities documented (Roberts, 1985). However, the presently isolated restriction endonucleases are still only a portion of those in existence. The finding of new restriction endonucleases is still required for the study of gene structure and recombinant DNA techniques.

We have screened for site-specific endonucleases in Streptomyces strains and found that different Streptomyces strains produce endonucleases with different specificities (Takahashi et al., 1979; Shimotsu et al., 1980). This report describes purification and characterization of a new site-specific endonuclease from Streptomyces caespitosus, named Scal.

MATERIALS AND METHODS

Bacterial strain and medium

Streptomyces caespitosus KCC-S-0438 was provided by Dr. A. Seino (Kaken Chemical Co., Tokyo, Japan). Cells were grown at 28 °C for 2 days with rotary shaking in a medium containing Nutrient broth (Difco), 10 g; yeast extract (Difco), 1 g; NaCl, 5 g; glucose, 2.5 g/l (pH 7.2), and were harvested by centrifugation.

DNA preparations

Phage DNAs, λC187Sam, 6X174 RFI and T4dC were prepared as described in previous papers (Takahashi et al., 1979; Shimotsu et al., 1980). pBR322 and pBR325 DNAs were purified by CsCl/ethidium bromide equilibrium centrifugation (Oka, 1978). Adenovirus 2 DNA and SV40 DNA were purchased from Bethesda Research Laboratories (Rockville, MD, U.S.A.). M13mp18 RF and single-stranded DNA were prepared from M13mp18 phage infected cells and the purified phage particles, respectively (Yanish-Perron et al., 1985). Synthetic nucleotide linker (5'-AAGTACTTT-3') was provided by Takara Shuzo Co. (Kyoto, Japan). A universal primer (3'-TGACCGCCAGCAAAATG-5') for the dideoxy chain-terminator method was provided by Dr. M. Kumagai (Yamasa Shoyu Co., Choshi, Japan).

Abbreviations used: bp, base pair; kb, kilobase; RF, replicative form; T4dC, cytosine-substituted T4 phage.

Enzymes

Restriction endonucleases Alul, HaeIII, HinfI, HpalI, HindIII, EcoRI, PstI and Rsal were purchased from Takara Shuzo Co. T4 polynucleotide kinase, T4 DNA ligase and Klenow fragment were purchased either from Takara Shuzo Co. or from Amersham (Japan) (Tokyo, Japan).

Enzyme assay

In a typical assay, 1–5 μl aliquots of column fractions were incubated in 20 μl of reaction mixture containing 0.5 μg of λC187Sam, DNA, 10 mm-Tris/HCl, pH 7.5, 100 mm-NaCl, 8 mm-MgCl2 and 10 mm-2-mercaptoethanol at 37 °C for 1 h. Reactions were terminated by the addition of 2 μl of 0.2 M-EDTA and heated at 65 °C for 5 min followed by rapid cooling. The cleavage products were analysed by electrophoresis on a horizontal agarose gel in 36 mm-Tris/32 mm-Na2PO4/1 mm-EDTA, pH 7.8 as described in previous papers (Takahashi et al., 1978; Takahashi & Saito, 1982). For separation of short DNA fragments, acrylamide-gel electrophoresis was used.

Purification of Scal endonuclease

Procedures for purification of Scal were essentially the same as the methods described previously (Takahashi et al., 1979; Shimotsu et al., 1980). Briefly, they were as follows. Cells were disrupted by sonication and, after high speed centrifugation, the supernatant was treated with streptomycin sulphate to a final concn. of 2% to remove nucleic acids. After removal of the precipitate, the supernatant was fractionated by (NH4)2SO4 and the 40–80% fraction, where most of the site-specific endonuclease activity was found, was dissolved in a minimal volume of PC buffer [10 mm-potassium phosphate (pH 7.4)/10 mm-2-mercaptoethanol/0.1 mm-EDTA/10%/ (v/v) glycerol] and dialysed against the same buffer. The solution was applied to a DEAE-cellulose (DE52, Whatman) column and developed with a linear gradient of 0–1.0 M-KCl in PC buffer. Active fractions, appearing at around 0.25 M-KCl, were pooled and dialysed against DE buffer [10 mm-Tris/HCl (pH 7.4)/10 mm-2-mercaptoethanol/0.1 mm-EDTA/10% (v/v)]
chromatography, cleaved with from Amersham. Determination of the properties of Scal endonuclease

RESULTS AND DISCUSSION

Purification and properties of Scal endonuclease

After DEAE-cellulose and DEAE-Seephacel column chromatography, the Scal enzyme preparation still contained a trace amount of non-specific nucleases, which were removed by heparin–Sepharose column chromatography. Elution of the adsorbed proteins by increasing potassium phosphate concentration was quite effective in removing the non-specific nucleases, since Scal activity appeared after the bulk of proteins had been eluted.

At this stage, the Scal preparation was essentially free of non-specific nucleases and contained no detectable phosphatase activity. This enzyme preparation was sufficient to use for characterization, since prolonged incubation of DNA with excess of enzyme preparation did not change the gel electrophoretic patterns. Comparison of the restriction patterns obtained from λ, adenovirus 2, T4dC and plasmid DNAs revealed that the specificity of the enzyme is different from those of known restriction endonucleases.

Scal was active in a broad pH range around 7.5. The optimal Mg++ concentration was 5–20 mM. Scal activity was stimulated by the addition of NaCl and the maximum activity was obtained at 0.1–0.13 M-NaCl.

Determination of recognition sequence of Scal

Digestion of pBR322, pBR325, adenovirus 2, and T4dC DNAs with Scal gave one, two, four and six at least eight bands, respectively, on a 1.0% agarose gel. No
cleavage site in φX174 RF DNA or SV40 DNA was found.

Based on these digestion data and on the tabularized sequencing data for several DNAs in Fuchs et al. (1980), it was deduced that the recognition sequence for Scal could be either 5'-AGTACT-3' or 5'-TCCGGA-3', since the other candidates do not occur in the cat gene or its flanking sequences in pBR325 (Prentki et al., 1981). The sequences AGTACT and TCCGGA occur in neither SV40 or φX174 RF DNA (Reddy et al., 1978; Sanger et al., 1978).

The palindromic AGTACT sequence occurs only once in pBR322 DNA at position 3845 (Sutcliffe, 1979). Also the sequence occurs in the cat gene on pBR325 DNA at position 5195 (Prentki et al., 1981) while the TCCGGA sequence occurs at a position 4776 which is only five bases apart from the EcoRI site. The Scal sites on pBR322 and pBR325 were analysed by digesting the DNA with Scal in combination with HindIII, PstI, EcoRI, AluI, HaeIII or HinfI, and comparing the gel patterns with those obtained when Scal was omitted. As a result, the unique Scal site on pBR322 was mapped on HaelII-A (0.50 kb), AluI-B (0.67 kb) and HinfI-A (1.6 kb) and the second Scal site on pBR325 was located in the cat gene at around position 5200 (Fig. 1). These results are consistent with the notion that Scal recognizes 5'AGTACT-3' sequences.

The proposed sequence 5'-AGTACT-3' for Scal contains the internal tetranucleotide 5'-GTAC-3', which is the recognition sequence for RsaI (Lynn et al., 1980). To see whether the Scal recognition sequence shares that of RsaI, double digestion products of pBR322 or pBR325 DNAs with RsaI and Scal were compared with fragments obtained with RsaI alone on agarose gel (Fig. 2), indicating that the cleavage sites for Scal are coincident with those for RsaI.

Based on these data, we have concluded that the recognition sequence for Scal is a palindromic hexanucleotide 5'-AGTACT-3'.

**Determination of cleavage site for Scal**

To determine the cleavage site of Scal endonuclease, a M13mp18 derivative into which had been inserted an AAGTACTT octanucleotide linker in the HincII (Salt) site was constructed and named M13mp18-SCA. Single-stranded M13mp18-SCA DNA was annealed with a primer and the primer-attached DNA was divided into five portions. Four portions were used for the dideoxy chain-terminator method (A, C, G and T ladders). The remaining portion was labelled without chain terminator and used for cleavage by Scal. The relevant sequences of M13mp-SCA and the Scal-cleaved fragment are shown in Fig. 3.

The Scal-cleaved product of the primer-extended M13mp18-SCA DNA was analysed by electrophoresis on a polyacrylamide sequencing gel (Fig. 4). The primer-

![Fig. 4. Sequencing gel analysis of Scal-cleaved DNA fragment](image)

Lane 1, Scal-cleaved fragment of the primer-extended M13mp18-SCA DNA; lanes 2–5 correspond to A, C, G and T chain-terminated ladders, respectively. The arrow in lane 1 shows a band produced by Scal cleavage. The arrow on the right-hand side indicates the site attacked by Scal.

The arrow indicates the Scal cleaving point.
Thus we conclude that ScaI recognizes a hexanucleotide palindromic sequence, 5'-AGT↓ACT-3', and cleaves it in the middle, producing blunt-ended fragments as indicated by the arrows.

The procedure used for ScaI cleavage site determination is a modification of the method described by Brown & Smith (1980). Now that we can use various types of M13mp vectors containing polylinkers for the dideoxy sequencing, and also easily obtain synthetic oligonucleotides, the procedure described in this report will be useful for determination of site-specific endonucleases. The reliability of the method was checked by using other well-known enzymes such as EcoRI, BamHI and PstI using the same DNA.

This work was supported in part by a Grant-in-Aid for Scientific Research and for Special Project Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES


Received 17 June 1985/22 July 1985; accepted 31 July 1985