Eco27kI, a new isoschizomer of *AvaI* from *Escherichia coli*

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A small collection (108) of *Escherichia coli* strains isolated in Kiev was surveyed for type II restriction endonucleases. 95 strains produced these enzymes; among them *Eco27kI*, a new isoschizomer of *AvaI* (1), recognized the palindromic sequence 5'-C/PyCGPuG-3'.

Eco27kI was purified by using DEAE-cellulose (Whatman DE52), phosphocellulose (Whatman PII) and ssDNA-agarose (2) columns. The enzyme eluted out at the flow through DE52 column at 150 mM NaCl and through two others at 50 mM NaCl was loaded onto DE52 column for rechromatography and hydroxyapatite (Serva HA-Ultragel) column chromatography with an intermediate 30–45% ammonium sulphate concentration/fractionation step and by gel-filtration on AcA44 Ultrogel (IBF biotechnics). *Eco29kI* activity eluted at 200 mM NaCl from DE52 and at 100 mM phosphate from HA-Ultragel. The enzyme was pure enough for 100 fold over-digestion on DNA.

The fragments produced from lambda DNA by *Eco27kI* are shown in Figure 1. The fragment profile of lambda DNA is identical to the one produced by *AvaI*. A double digest between *Eco27kI* and *AvaI* on bacteriophage lambda DNA confirmed that these enzymes are isoschizomers.

The position of phosphodiester bond cleavage within the recognition site was determined by examination of a primed synthesis reaction. Sequencing reactions were performed as described by Sanger *et al.* (3). An M13tg130 DNA template containing an *AvaI* (*SmaI*) site and a primer (22 bases) were used to synthesize double-stranded DNA through the *AvaI* site. The extended DNA was digested with *Eco27kI* and *AvaI* separately. Aliquots of the digested products were run in lanes (Figure 2) next to a sequencing ladder produced with the same primer and template. The results show that *Eco29kI* produces a fragment which co-migrates with the first 5' C within the recognition sequence CCCGGG, essentially as *AvaI*. Thus, *Eco27kI* and *AvaI* are true isoschizomers. All known isoschizomers of *AvaI* cut DNA substrates in the same point (4).

The optimal reaction conditions of *Eco27kI* are: 50–100 mM NaCl (75–100 mM KCl), 10 mM Tris-HCl (pH 7.5–8.5), 10 mM MgCl₂, 37°C.

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Figure 1. Digest of Lambda DNA: 1 — *NruI*; 2 — *Eco27kI*; 3 — *Eco27kI* + *AvaI*; 4 — *AvaI*; 5 — *CfrBI* (*StyI*).

Figure 2. Determination of *Eco27kI* cleavage site.

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