Isolation and identification of restriction endonuclease, *SeI* from a cyanobacterium, *Synechococcus elongatus*

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A restriction endonuclease, *SeI* has been isolated from a thermophilic and unicellular cyanobacterium, *Synechococcus elongatus* (1). *SeI*, an isoschizomer of *FnuDII* (2), recognizes and cleaves at the sequence, 5'-↓CGCG-3'.

The enzyme was purified using the following column chromatographic steps: 1) phosphocellulose, 2) Heparin-Sephrose, 3) AH-Sephrose. Optimal conditions for *SeI* activity were 40 mM NaCl, 10 mM MgCl₂, pH 6.5, and 50°C of reaction temperature. Magnesium ion was essential to specific cleavage by *SeI*.

The recognition site of *SeI* was predicted by electrophoresis analysis of digestion products by *SeI*. Figure 1 indicates results of electrophoresis of λ-DNA and pUC18 digested by *SeI* and *AccII* which was also the isoschizomer of *FnuDII* (2). The electrophoresis patterns made by two kinds of the enzymes were quite similar. Determination of *SeI*-cleavage positions according to primer extension method (3) is described in Figure 2. The cleaved product by *SeI* resulted in a single band (lanes 1 and 2 of Figure 2) which comigrates with 5'A in the sequence 5'ACGCG3'. Furthermore, by the addition of T4 DNA polymerase to the *SeI*-cleaved fragment (lane 3 of Figure 2), 4 base extensions were observed in the ³²P-labeled strand in comparison with lanes 1 and 2 therein. These results indicate that *SeI* recognized 5'↓CGCG3' and made tetranucleotide overhang for 5'-terminal extensions.

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Figure 1. Comparison of the products of *SeI*-digestion with that of *AccII* digestion. Lanes: 1, λDNA digested with *SeI*; 2, λDNA digested with *AccII*; 3, pUC18 digested with *SeI*; 4, pUC18 digested with *AccII*.

Figure 2. Determination of *SeI* cleavage positions. First, substrate DNA for *SeI* was synthesized by enzymatic extension method using the ³²P-labeled M13-universal primer with the single stranded M13mp18-derivative containing the *SeI* cleavage site as template DNA. Then, the synthesized double strand DNA was treated by *SeI*. The *SeI*-treated DNA was divided into two portions; the one portion was applied on lanes 1 and 2 after denaturation, and the other was further treated with T4 DNA polymerase, denatured and applied on lane 3. Lanes G, A, T and C were DNA ladder patterns made by M13-dideoxy method when the same primer and template DNA as for synthesis of the substrate DNA were used.