Characterization of *NgoAIII*, an isoschizomer of *SstII* from a strain of *Neisseria gonorrhoea*

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A type II restriction endonuclease, *NgoAIII*, has been isolated from a strain of *Neisseria gonorrhoea*. *NgoAIII* recognizes the sequence 5'CCGC↓GG3' producing a 2-base 3'-extension. The fragments generated after the cleavage of adenovirus 2 DNA (Ad2 DNA) by *NgoAIII* are shown in Figure 1. An identical fragment profile is obtained when Ad2 DNA is cleaved with *SstII* (Fig. 1), which recognizes the same sequence. Therefore, the cleavage site of *NgoAIII* was determined and compared with that of *SstII*.

The position of phosphodiester bond cleavage within the recognition site was determined by the method of Brown and Smith (1). Sequencing reactions were performed as described by Sanger, et.al. (2). A DNA fragment containing an *SstII* site was introduced into M13mp19 for cleavage-site determination. Single-stranded DNA template containing the *SstII* site was used to synthesize double-stranded DNA through the *SstII* site using BRL universal primer. The extended DNA was cleaved with *NgoAIII* and *SstII* separately. Aliquots of the digested products were run on a sequencing gel (Fig. 2) next to DNA sequencing reaction products produced with the same template and primer. The results show that both *NgoAIII* and *SstII* produced a fragment which co-migrates with the 3'C within the recognition sequence 5'CCGCGG3'. Treatment with Klenow fragment or T4 DNA polymerase in the presence of all four deoxyribonucleotides, subsequent to *NgoAIII* or *SstII* digestion, shifted the migration of the fragments to a position corresponding to the second 5'C, consistent with the elimination of two nucleotides. These results demonstrate that both *NgoAIII* and *SstII* cleave at the same site within the sequence. In addition, Ad2 DNA fragments generated by *NgoAIII* digestion can be cloned into a *SstII*-cleaved vector and all cloned fragments are recovered by digestion with either *SstII* or *NgoAIII*. Moreover, DNA isolated from *NgoAIII* methylase producing strains is resistant to *SstII* cleavage demonstrating that *NgoAIII* methylase protects against *SstII* restriction.

Figure 1

Figure 2

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References:

1. Brown, N.L. and Smith, M. (1980) Methods Enzymol. **65**, 391-404.
2. Sanger, et.al. (1980) J. Mol. Biol. **143**, 161-178.