*Mha*AI, a novel isoschizomer of *Pst*I from *Mycobacterium habana* recognizing 5′-CTGCA/G-3′

Sandeep Shankar and Anil K.Tyagi*
Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India

Submitted April 13, 1992

We have isolated a novel class II restriction endonuclease, *Mha*AI, from *Mycobacterium habana* strain 206 which recognizes sequence 5′-CTGCA/G-3′ and generates 3′ protruding fragments.

Unlike its isoschizomer *Pst*I, *Mha*AI did not exhibit star activity even at glycerol concentrations as high as 35% and at units/ug DNA ratios greater than 300. *Mha*AI was fully active in low salt (10 mM Tris pH 8.0, 10 mM $MgCl_2$) in comparison to *Pst*I, which requires NaCl at a concentration of 50−100 mM for optimal activity. *Mha*AI was also found equally active in 10 to 200 mM concentration range of KCl as well as $MgCl_2$. The enzyme, however, was inhibited by NaCl concentrations greater than 50 mM.

Comparison of cleavage patterns experimentally obtained with *Mha*AI on standard DNAs of known nucleotide sequence; bacteriophage lambda, adenovirus-2, pGEM3Z, SV40 and phiX174 with computer derived mapping data predicted the sequence 5′-CTGCAG-3′ (Figure 1).

The cut position within the *Mha*AI recognition site was determined according to the enzymatic sequencing approach (1). A pUC8 recombinant with an insert containing an *Mha*AI site was used for enzymatic sequencing reactions starting with a primer approximately 120 base pairs from the recognition site. In a parallel reaction, the same primer end-labelled with [gamma-^{32}P] ATP was annealed to the template and the labelled primer was extended through the *Mha*AI site by treatment with Sequenase version 2.0 (2, 3) in the presence of all four dNTPs. The double stranded DNA was used as substrate for *Mha*AI to produce 5′ end labelled DNA fragments comparable to the sequencing ladder. Samples were analysed without and with (−/+) further incubation with T4 DNA polymerase and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2).

From the mapping and sequencing data, the specificity of *Mha*AI is concluded as:

5′ CTGCA/G 3′
3′ G/ACGTC 5′

ACKNOWLEDGEMENT

We are thankful to Dr Naresh Kumar Jain, New Delhi Tuberculosis Centre, New Delhi for providing us with the *Mycobacterium habana* strain 206.

REFERENCES

1. Brown,N.L. and Smith,M. (1980) *Methods Enzymol.* **65**, 391−404.
2. Tabor,S. and Richardson,C.C. (1987) *Proc. Nat. Acad. Sci. USA* **84**, 4767−4771.
3. Tabor,S. and Richardson,C.C. (1989) *J. Biol. Chem.* **264**, 6447−6458.

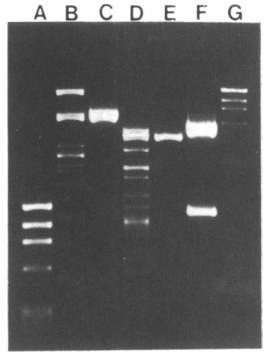

Figure 1. Lanes [A] and [G] MW markers (phiX174-*Hae*III fragments and bacteriophage lambda DNA-*Hin*dIII fragments respectively). Lanes [B] to [F] represent *Mha*AI digests on [B] bacteriophage lambda, [C] phiX174, [D] adenovirus-2, [E] pGEM3Z and [F] SV40 DNA.

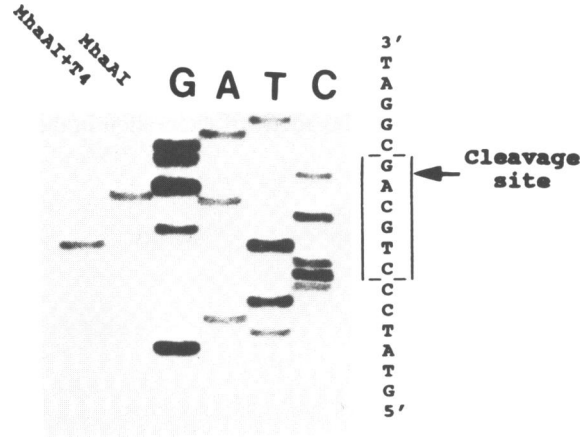

Figure 2. Determination of *Mha*AI cleavage position.

* To whom correspondence should be addressed