Restriction endonuclease *NciI* is not blocked by CpG methylation

Richard R. Meehan, Eugen Ulrich and Adrian P. Bird*

Institute of Cell and Molecular Biology, University of Edinburgh, Kings Buildings, Edinburgh EH9 3JR, UK

Received October 18, 1993; Accepted October 22, 1993

Vertebrate DNA is highly methylated at cytosines in the dinucleotide sequence CpG (1). Many restriction enzymes are unable to cleave DNA if their recognition sequences contain methylated CpG (2), and this property has been used to study the pattern of DNA methylation in higher eukaryotes (3). An enzyme that has recently been used in this way is *NciI* (recognition sequence CCSGG, where S is C or G). It was reported that this enzyme cannot cleave a region of the mouse actin promoter when the CpG in its recognition site is methylated on both strands, but can cleave when either one or both strands are unmethylated (4). As a result, *NciI* has been used to monitor the conversion of symmetrically methylated DNA to hemi-methylated DNA in a number of systems (4,5,6). Its general use for this purpose depends upon the idea that *NciI* cannot cleave any symmetrically methylated site.

We have digested symmetrically methylated and non-methylated DNA with *NciI*, and find that it can cleave both forms to completion, though at markedly different rates. Figure 1 shows the digestion patterns with various restriction enzymes of the plasmid pAdML in non-methylated and methylated forms. The plasmid was methylated with the cytosine DNA methyltransferase M.SssI which methylates all CpGs on both strands (7). As expected, *MspI* cut both forms of the plasmid, whereas its methylation-sensitive isoschizomer *HpaII* cut only the non-methylated form of the plasmid (lane 2). Since *NciI* sites must include *HpaII* sites, this result shows that all *NciI* sites are methylated at CpG. *NciI* at 40 units/ μ g DNA cut both the methylated and non-methylated plasmids to completion (lanes 10). At lower enzyme concentrations (2.5–30 units/ μ g of DNA), the methylated plasmid was partially cut, while the non-methylated form was still cut to completion (lanes 4–9). This suggests that symmetrical methylation at CpG inhibits the rate of digestion by *NciI*, but does not block its action. The difference in the rate of digestion appears to be at least 20 fold.

We obtained equivalent results using as substrate a large fragment of *Xenopus* ribosomal DNA that had been methylated at all *NciI* sites with M.*HpaII* methyltransferase. Methylated and non-methylated samples were cut identically by *NciI*, although the former was cleaved more slowly (data not shown). While *NciI* sites in certain sequence contexts may be completely blocked by methylation (4), our data indicate that the great majority of symmetrically methylated sites are in fact cuttable by this enzyme. Thus *NciI* is not suitable for routine use as a methylation-sensitive restriction enzyme.

Figure 1. Digestion of methylated and non-methylated plasmid DNA with the restriction enzyme *NciI*. 1 μ g of the plasmid pAdML (8) in its non-methylated form (A) and its methylated form (B) was digested with various restriction enzymes. All reactions were carried out according to the manufacturers recommendations (Boehringer Mannheim), incubated for 1 hour at 37°C and run on a 1% TAE agarose gel. Plasmid pAdML was methylated at all its CpGs using M.SssI methyltransferase according to the manufacturers recommendations (New England Biolabs). The completion of the methylation reaction was monitored by testing with the methylation-sensitive restriction enzymes *HpaII* and *CfoI* (not shown). Lanes show digests of DNA after incubation with 1, no enzyme; 2, *HpaII*; 3, *MspI*; 4, 2.5 units *NciI*; 5, 5 units *NciI*; 6, 7.5 units *NciI*; 7, 10 units *NciI*; 8, 20 units *NciI*; 9, 30 units *NciI*; 10, 40 units *NciI*. 'M', marker lane containing 1 μ g of the 1 kb ladder (Gibco BRL). Sizes are in kilobases. C shows the location of the *NciI* sites in pAdML.

* To whom correspondence should be addressed

ACKNOWLEDGEMENTS

This work was supported by The Wellcome Trust, the Imperial Cancer Research Fund. E.U. is a Wellcome Research Assistant, and R.R.M. is a member of the ICRF Epigenetics Laboratory. A.P.B. is a Howard Hughes International Research Scholar.

REFERENCES

1. Razin, A. and Riggs, A.D. (1980) *Science* **210**, 604–610.
2. Nelson, N., Raschke, E. and McClelland, M. (1993) *Nucleic Acids Res.* **21**, 3139–3154.
3. Cooper, D.N., Taggart, M.H. and Bird, A.P. (1983) *Nucleic Acids Res.* **11**, 647–658.
4. Paroush, Z., Keshet, I., Yisraeli, J. and Cedar, H. (1990) *Cell* **63**, 1229–1237.
5. Sasaki, T., Hansen, S. and Gartler, S.M. (1992) *Mol. Cell. Biol.* **12**, 3819–3826.
6. Cooper, G.E., Bishop, P.L. and Turker, M.S. (1993) *Somatic Cell Mol. Gen.* **19**, 221–229.
7. Renbaum, P., Abrahamove, D., Fainsod, A., Wilson, G., Rottem, S. and Razin, A. (1990) *Nucleic Acids Res.* **18**, 1145–1152.
8. Heiermann, R. and Pongs, O. (1985) *Nucleic Acids Res.* **13**, 2709–2730.