*Bce*83I, a restriction endonuclease from *Bacillus cereus* 83 which recognizes novel nonpalindromic sequence 5′-CTTGAG-3′ and is stimulated by S-adenosylmethionine

Nadezhda N.Matvienko, Vladimir M.Kramarov, Leonid Yu.Ivanov[1] and Nicholas I.Matvienko[1]*
Russian Center of Molecular Diagnostics and Therapy, Simferopolsky Bvd 8, 113149 Moscow and [1]Institute of Protein Research, Academy of Sciences of Russia, 142292 Pushchino, Moscow Region, Russia

Submitted March 3, 1992

Restriction endonuclease *Bce*83I has been purified by chromatography on blue-sepharose and hydroxyapatite. It recognizes 4, 6, 5, 13 and more than 20 sites on pUC18, pBR322, M13mp18, λ and T7 DNA, respectively. Double digestion of pUC18 with *Bce*83I and *Pvu*II, *Hin*dIII, *Cfr*10I and *Bme*216I (isoschizomer *Ava*II) showed that the *Bce*83I sites localized to the position 912, 1179, 1451, 2319, where the same sequence CTTGAG or the complementary to it are located. The numbers of cleavage sites and lengths of the DNA fragments after treatment of the M13mp18, pBR322 and λDNA are consistent with this recognition site. To identify the points of cleavages the recombinant phase M13tg130 with the λ*Hin*dIII fragment with coordinates 37459−37584 was constructed. The cleavage points were determined by cleavage of a primer−synthesis reaction (1). The cleaved product resulted in a predominant band comigrated with G at a distance of 14 nucleotides from the recognition site. At addition of DNA polymerase the band appeared two nucleotides lower (Figure 1). These results indicate that *Bce*83I recognizes the site 5′-CTTGAG-16N↓N-3′ and cleaves DNA

3′-GAACTC-14N↓N-5′

as indicated by arrows. The enzyme is activated more than 30-fold by S-adenosylmethionine (Figure 2). Consequently it belongs to the type IV of restriction endonucleases (2).

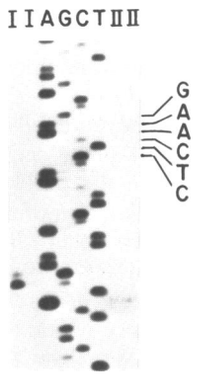

Figure 1. Determination of the Bce83I cleavage site. I: The template of primed-synthesis reaction was cleaved with *Bce*83I, II: The product from line I was treated with DNA polymerase; A, G, T, C: The sequence ladder through the *Bce*83I recognition sequence, using the termination method.

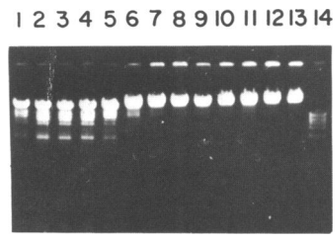

Figure 2. S-adenosylmethonine requirement of *Bce*83I endonuclease. Cleavages of λDNA. With addition of SAM into the incubation mixture: 1 — 100 μM; 2 — 20 μM; 3 — 4 μM; 4 — 0.8 μM; 5 — 160 nM; 6 — 32 nM; 7 — 6.4 nM; 8 — 1.2 nM; 9 — 250 pM; 10 — 50 pM; 11 — 10 pM; 12 — 2 pM; 13 — without SAM; 14 — SP6*Hin*dIII fragment (marker of molecular weights).

REFERENCES

1. Brown,N.L. and Smith,M. (1980) *Methods Enzymol.* **65**, 391−404.
2. Petrušyté,M. Bitinaité,J., Menkevicius,S., Klimašauskas,S., Butkus,V. and Janulaitis,A. (1988) *Gene* **74**, 89−91.

* To whom correspondence should be addressed