New restriction endonuclease CviRI cleaves DNA at TG/CA sequences

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ABSTRACT

A new type II restriction endonuclease, CviRI, was isolated from virus XZ-6E infected chlorella cells. CviRI is the first restriction endonuclease to recognize the sequence 5'-TGCA-3' and cleaves DNA between the G and C residues to produce blunt-end termini. Methylation of the adenine or cytosine in 5'-TGCA-3' sequences prevents CviRI cleavage. Due to its sequence specificity, CviRI may be especially useful for detecting mutant alleles of many heritable human genetic diseases.

INTRODUCTION

A DNA site-specific (restriction) endonuclease, CviRI, was purified from chlorella infected with the large dsDNA-containing virus XZ-6E. CviRI is the first restriction endonuclease to recognize the sequence TGCA; it cleaves DNA between the G and C residues to produce blunt-ended termini.

MATERIALS AND METHODS

Virus XZ-6E was isolated from a freshwater lake in Xuzhou, China (1). *Chlorella* strain NC64A (2×10^7 cells/ml) was infected with XZ-6E (multiplicity of infection of 5), infected cells were harvested at 4 hr post-infection, and cell extracts were prepared as previously described (2). Clarified extracts were applied in succession to the following chromatography columns and eluted as indicated: Heparin sepharose CL-6B (Pharmacia), 0.3 to 1.0 M NaCl linear gradient, pH 7.5 (CviRI elutes ca. 0.7 M); Blue sepharose CL-6B (Pharmacia), 0.3 to 1.2 M NaCl linear gradient, pH 7.5, (CviRI elutes ca. 0.8 M); hydroxylapatite (BioRad HTP), 0.01 to 1.0 M potassium phosphate linear gradient, pH 7.4 (CviRI elutes ca. 0.6 M). Fractions with endonuclease activity were combined, dialyzed against [10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol] and stored at -20°C . Enzyme activity was

assayed for 1–2 hr at 23°C in 25 μl reactions containing [10 mM Tris-HCl (pH 7.8), 10 mM MgCl_2 , 150 mM KCl, 100 $\mu\text{g/ml}$ bovine serum albumin], and 1 μg of DNA. The reactions were stopped with 12 μl of 50 mM EDTA, 50% glycerol, and 0.1% bromophenol blue, followed by electrophoresis on 2.0–2.5% agarose gels containing 0.25 $\mu\text{g/ml}$ ethidium bromide.

RESULTS AND DISCUSSION

CviRI cleaves pUC19 (Fig. 1A, lane 1), pBR322, and phage lambda DNAs into well defined fragments. Computer search analysis ('FINDPATTERNS', University of Wisconsin GCG program package) indicated that the sizes of CviRI restriction fragments differed from those expected from all known palindromic four base pair recognition restriction endonucleases. However, the sizes of these fragments were consistent with cleavage at 5'-TGCA-3' sequences. As shown in Fig. 1B, CviRI cleaves between the G and C residues of TGCA sequences.

CviRI did not cleave $\text{TGC}^{\text{m}}\text{A}$ -modified virus XZ-6E DNA, plasmid pXZ-6E.14 DNA, which expresses the M.CviRI adenine methyltransferase gene (3), or phage Xp12 DNA, which contains 5-methylcytosine in place of cytosine (4). Furthermore, CviRI did not cleave $\begin{smallmatrix} 5'\text{-TGCA-3'} \\ 3'\text{-ACGT-5'} \end{smallmatrix}$ hemi-methylated DNA (Fig. 1A, lane 2). Thus CviRI cleaves unmodified TGCA sequences, but not methylated $\text{TGC}^{\text{m}}\text{A}$ or $\text{TG}^{\text{m}}\text{CA}$ sequences.

CviRI may be especially useful in the diagnosis of mutant alleles for human genetic diseases because: (i) Deamination-induced $5^{\text{m}}\text{CG} \rightarrow \text{TG}$ transitions are about forty times more frequent in mammalian DNA than other point mutations (5,6). (ii) The TG dinucleotide occurs twice in the palindromic $\begin{smallmatrix} 5'\text{-TGCA-3'} \\ 3'\text{-ACGT-5'} \end{smallmatrix}$ sequence. Consequently, mutant genes for many 3'-ACGT-5' human genetic diseases contain CviRI sites, whereas the corresponding wild type genes do not. Examples include: adenosine deaminase (severe combined immunodeficiency disease) (7), α -1 procollagen (osteogenesis imperfecta) (8), ApoE (type III hyperlipoproteinemia) (9), p53 tumor suppressor (10), and C1 serpin (11) genes.

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Figure 1. (A) CviRI cleavage products of pUC19 DNA. The CviRI cleavage site at position 2367 in pUC19 DNA overlaps an M.EcoK modification sequence $Am^mACN_6G\ TGC$. Therefore, pUC19 DNA prepared from an *E.coli hsd⁺* strain is hemimethylated at this site and this site is resistant to CviRI cleavage (lane 2). In contrast, pUC19 DNA prepared from an *hsd⁻* *E.coli* strain is cleaved at position 2367 (lane 1). (B) CviRI (R1 lanes) cleaves between the G and C in 5'-TGCA-3' sequences. Radiolabelled pBluescript KS(-) or KS(+) ssDNA-primed synthesis products were cleaved with CviRI and the fragments were compared with G, A, T, and C dideoxy chain termination sequencing products of pBluescript KS(-) or KS(+) ss-DNA templates, respectively.

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