Note

Characterization of Restriction Endonuclease BdiSI, an Isoschizomer of SfeI, from Bacteroides distasonis Strain S-7†

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To obtain more useful restriction endonucleases, we screened 128 strains of anaerobic bacteria and found that a strain of Bacteroides distasonis Eggerth and Gagnon 1933 produces a restriction endonuclease named BdiSI.1) However, this restriction endonuclease has been known to be an isoschizomer of SfeI.2) This paper describes the purification and properties of this restriction endonuclease.

Cells of Bacteroides distasonis strain S-7 (85 g, wet weight), cultivated anaerobically at 37°C for 24 hr in a medium containing 0.4% casamino acids, 0.3% peptone, 1.0% glucose, 0.15% sodium chloride, 0.1% Tween 80 and 0.04% L-cysteine, pH 7.2, were disrupted by passing through a French pressure cell (Ohtake Works Co.) at 1500-1700 kg/cm². Nucleic acids were removed from the cell extracts by 1% streptomycin treatment, and then the solution was adjusted to 60% (NH₄)₂SO₄ (w/v) by addition of solid (NH₄)₂SO₄. Precipitates were collected and dissolved in buffer A (50 mm Tris–HCl, 7 mm 2-mercaptoethanol and 7 mm MgCl₂, pH 7.5). The solution was dialyzed overnight against buffer A. The dialyzed solution (240 ml) was used for HPLC on a DEAE-Toyopearl pak 650M column (2.2 x 20 cm). The enzyme was eluted with a linear gradient of buffer A containing 0-400 mM NaCl (90 ml in total). The enzyme was recovered at 60-250 mM NaCl. To the enzyme solution, glycerol was added at a final concentration of 10% (v/v). The solution (420 ml) was put onto a TSKgel Heparin-5PW Glass column (0.8 x 7.0 cm). A fraction containing the enzyme was obtained by elution with buffer B (30 ml in total, 50 mM Tris–HCl, 7 mM 2-mercaptoethanol, 7 mM MgCl₂ and 10% glycerol, pH 7.5) with a linear gradient of 200-600 mM NaCl. The enzyme was eluted at 420-600 mM NaCl, and the enzyme solution was concentrated with Centricut (Biofield Corp.). The concentrated enzyme solution (4 ml) was chromatographed by HPLC on a TSKgel G3000SW Glass column (0.8 x 30 cm) and eluted with buffer B containing 300 mM NaCl at 0.8 ml/min. The enzyme was recovered in a fraction flowing out at retention times of 14.3-17.5 min. Active fractions were combined and dialyzed overnight against buffer B. The dialyzed solution was put onto a TSKgel DEAE-5PW Glass column (0.8 x 7.0 cm) and eluted with a linear gradient of buffer B (20 ml in total) containing

Table I. Purification of Restriction Endonuclease BdiSI from Bacteroides distasonis Strain S-7

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (unit)</th>
<th>Specific activity (unit/mg)</th>
<th>Recovery (%)</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>7,400</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>5,500</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>3,300</td>
<td>110,000</td>
<td>33</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>DEAE-Toyopearl pak</td>
<td>480</td>
<td>97,000</td>
<td>200</td>
<td>88</td>
<td>6.1</td>
</tr>
<tr>
<td>Heparin-6PW</td>
<td>4.6</td>
<td>15,000</td>
<td>3,300</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>G3000SW</td>
<td>0.39</td>
<td>9,300</td>
<td>24,000</td>
<td>8.5</td>
<td>730</td>
</tr>
<tr>
<td>DEAE-5PW</td>
<td>0.021</td>
<td>1,200</td>
<td>57,000</td>
<td>1.1</td>
<td>1,700</td>
</tr>
</tbody>
</table>

One unit of the enzyme activity was defined as the amount of the enzyme required for complete digestion of 1.0 μg of λ DNA at 37°C in 60 min under the assay conditions described in the text. Protein was measured by the absorbances at 280 nm and 260 nm.

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Abbreviations: HPLC, high performance liquid chromatography; bp, base pairs.
Fig. 1. Relative Molecular Mass Measurement of Purified Enzyme from Bacteroides distasonis Strain S-7.

The purified enzyme was measured by gel filtration on a TSKgel G3000SW Glass column (0.8 x 30 cm). A buffer (50 mM Tris–HCl, 17 mM 2-mercaptoethanol, 7 mM MgCl₂, 300 mM NaCl and 10% glycerol (v/v), pH 7.5) was used into the column as the eluent at a flow rate of 0.8 ml/min. Ferritin (440,000 daltons), aldolase (158,000), ovalbumin (43,000) and ribonuclease A (13,700) were used as reference standards.

0–250 mM NaCl. The enzyme was recovered at 108–117 mM NaCl. The purity and recovery of the enzyme are summarized in Table I.

The purified enzyme was homogeneous on polyacrylamide gel disc electrophoresis. The relative molecular mass of the enzyme was 25,000 daltons by gel filtration using ferritin (440,000 daltons), aldolase (158,000), ovalbumin (43,000) and ribonuclease A (13,700) as reference standards (Fig. 1).

Lambda DNA was digested with the purified enzyme at 37°C for 0 to 24 hr in reaction mixtures (50 µl) containing 1.0 µg DNA, 10 mM Tris–HCl, 7 mM 2-mercaptoethanol and 7 mM MgCl₂, pH 8.0. No remarkable changes were observed in the digestion patterns of 24-hr digestion. These digestion patterns suggested that the purified enzyme preparation does not contain any other nucleases. Activity of the purified enzyme preparation does not contain any other nucleases. Activity of the purified enzyme preparation (1.0 unit) was measured at different temperatures from 30°C to 50°C, and the effects of pH on the enzyme reaction were examined from pH 4.0 to 10.0 with λ DNA (1.0 µg) as the substrate in a 50-µl mixture containing 10 mM Na-acetate, pH 4.0 (or piperazine–HCl, pH 5.0; histidine–HCl, pH 6.0; imidazole–HCl, pH 7.0; Tris–HCl, pH 8.0; monoethanolamine–HCl, pH 9.0; triethylamine–HCl, pH 10.0), 7 mM 2-mercaptoethanol and 7 mM MgCl₂. The optimum temperature and pH for the enzyme were 37°C and pH 8.0 under the above conditions. By the similar experiments, we found that the enzyme is stable at 40°C at pH 7.5 for 5 min and between pHs 6.0 and 8.0 for 24 hr at 4°C. Although we also examined the effects of NaCl concentration under the same conditions, the enzyme was not affected by this compound.

The enzyme cleaved M13mp18 RF I, φX174 RF I, SV40 and pBR322 DNAs at ≥ 5, 5, ≥ 5 and 4 sites, respectively. The enzyme produced four DNA fragments (about 190, 680, 890 and 2600 bp) from pBR322 DNA. Palindromic nucleotide sequences satisfying these data are 5'-C-T-(A or G)-(C or T)-A-G-3' sequences only in the data of Fuchs et al. When the pBR322 DNA was double-digested with this enzyme and Psfl, no great changes were found. These data indicated that the enzyme recognizes the same sequence as PstI does.

To identify the recognition sequences and cleavage sites, 25 µg of pBR322 DNA was digested with 60 units of the purified enzyme preparation at 37°C and pH 8.0 for 2 hr under the indicated conditions (250 µl). The resulting linear DNA molecules were dephosphorylated with a bacterial alkaline phosphatase (E. coli C75, 1.0 unit) and labeled at the 5'-termini with [γ-³²P]ATP using T4 polynucleotide kinase. The labeled DNA molecules were treated with HindIII, and the resultant three fragments (109, 191 and 783 bp) were separated by 5% polyacrylamide gel disc electrophoresis. All the 5'-terminal nucleotides of the fragments identified by the procedure of Ikawa et al. were T. Two of the three fragments (109 and 783 bp) were sequenced by the method of Maxam and Gilbert. The gel pattern of the small fragment (109 bp) was 5'-T-A-C-A-G-C-A-T-C-A-G-3' from coordinate 142 in the 3'-direction (coordinate 139 and further).

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5'-C-T-(A or G)-(T or C)-A-G-3'
3'-G-A-(T or C)-(A or G)-T-C-5'

and cuts at the positions indicated by the arrows.

In a preliminary report, we named BdIl for the restriction endonuclease from B. distasonis strain S-7. However, we have found that the name BdIl was already used by Kim and Rho for the enzyme from a strain of Brevibacterium diversatum, an isoschizomer of Clal which recognizes S'-A-T-C-G-A-T-5' sequence and cuts between T and C. Kim and Rho's enzyme cleaves a different DNA sequence from ours. In this report, we have renamed our enzyme BdISI.

Our enzyme does not require NaCl for its reaction. In contrast, SfEl requires NaCl. Since the relative molecular mass of BdISI (an isochizomer of SfEl) is small (25,000 daltons), the enzyme is functional in a monomeric structure. However, no detailed physicochemical properties of SfEl have been measured. It is of interest whether SfEl has the same monomeric structure as BdISI or not.

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References