Note

The Restriction Endonuclease Gceinl of *Gluconobacter cerinus* IFO 3260, an Isoschizomer of *BamHI*, Has a Monomeric Structure†

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Type II restriction endonucleases, which are indispensable for gene manipulation and gene analysis, are widely found in the Procaryotae such as bacteria and cyanobacteria.† During the course of our studies, we reported a new restriction endonuclease ApaLI from cells of *Acetobacter pasteurianus* IFO 13753.‡.§ We have found that the restriction endonuclease Gceinl, an isoschizomer of *BamHI*, has a monomeric structure different from the *BamHI* endonuclease. This paper is concerned with the purification and properties of the restriction endonuclease Gceinl of *Gluconobacter cerinus* (ex Asai 1935) Yamada and Akita 1984† IFO 3260.*1

The restriction endonuclease Gceinl was purified from cell-free extracts (1.5 l) prepared from 200 g of intact cells, wet weight) of *G. cerinus* IFO 3260 by streptomycin treatment, ammonium sulfate fractionation, column chromatographies on heparin-Sepharose CL-6B and DEAE-Sepharose CL-6B and FPLC on Mono Q HR 5/5 (Pharmacia)† (Table 1).

The purified enzyme was homogeneous on SDS-polyacrylamide gel slab electrophoresis, and the relative

Table I. Purification of the Restriction Endonuclease Gceinl of *Gluconobacter cerinus* IFO 3260

<table>
<thead>
<tr>
<th>Purification 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-free extract</td>
<td>8,700</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Streptomycin (1%)</td>
<td>5,600</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ammonium sulfate (25–60%)</td>
<td>2,700</td>
<td>49,000</td>
<td>18</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Heparin-Sepharose CL-6B†</td>
<td>57</td>
<td>24,000</td>
<td>420</td>
<td>49</td>
<td>23</td>
</tr>
<tr>
<td>DEAE-Sepharose CL-6B‡</td>
<td>1.4</td>
<td>10,000</td>
<td>7,100</td>
<td>20</td>
<td>400</td>
</tr>
<tr>
<td>Mono Q HR 5/5§</td>
<td>0.81</td>
<td>7,500</td>
<td>9,300</td>
<td>15</td>
<td>520</td>
</tr>
</tbody>
</table>

* The sample (150 ml) was put onto the column (1.5 x 20 cm, 30 ml), and the column was rinsed with 300 ml of buffer D (50 mM Tris-HCl, 7 mM 2-mercaptoethanol, 7 mM MgCl₂, 100 mM NaCl and 5% glycerol, v/v, pH 7.2). The endonuclease activity was recovered in fractions (5 ml each) 110–122 (corresponding to 0.15–0.30 M NaCl) when developed with a linear gradient of buffer D containing 0.1–0.6 M NaCl (300 ml in total).

† The sample (72 ml) was put onto the column (1.3 x 20 cm, 26 ml), and the column was rinsed with 75 ml of buffer E (50 mM Tris–HCl, 7 mM 2-mercaptoethanol, 7 mM MgCl₂ and 5% glycerol, v/v, pH 7.2). The endonuclease activity was recovered in fractions (2.5 ml each) 92–95 (corresponding to 0.20–0.25 M NaCl) when developed with a linear gradient of buffer E containing 0–1.0 M NaCl (260 ml in total).

‡ The sample (11 ml) was put onto the column (0.5 x 5.0 cm, FPLC, Pharmacia), and the column was rinsed with 15 ml of buffer F (50 mM Tris–HCl, 7 mM 2-mercaptoethanol and 7 mM MgCl₂, pH 7.2). The endonuclease activity was recovered in fractions (0.5 ml each) 31–32 (corresponding to 0.51–0.57 M NaCl) when developed with a linear gradient of buffer F containing 0–1.0 M NaCl (15 ml in total).

§ One unit of the enzyme activity (Gceinl) was defined as the amount of the enzyme required to completely digest 1.0 µg of λDNA in 60 min at 37°C under the optimum reaction conditions described in the text.

† This constitutes Part VIII of a series entitled "Studies on Restriction Endonucleases of Acetic Acid Bacteria and Allied Organisms." For Part VII, see ref. 4.

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Abbreviations: SDS, sodium dodecyl sulfate; bp, base pairs; FPLC, fast protein liquid chromatography.

*1 This strain was once classified as *Gluconobacter oxydans* (Henneberg 1897) De Ley 1961 subsp. *industries* (Henneberg 1898) De Ley and Fruet 1974.6)
The purified enzyme (15 μg) was run and stained with 0.1% Coomassie Brilliant Blue R-250. The electrophoresis was done on a 12.5% SDS-polyacrylamide gel slab (15 x 18 x 0.2 cm), which was standardized with bovine albumin (66,000 daltons), egg albumin (45,000 daltons), trypsinogen (24,000 daltons), β-lactoglobulin (18,400 daltons) and lysozyme (14,300 daltons). Small amounts of the standard proteins flowed into the sample lane. The relative molecular mass of the purified enzyme (GceinI) was calculated to be 28,000 daltons.

The relative molecular mass was 28,000 daltons (Fig. 1). The relative molecular mass measurement by gel filtration on Superose 12 HR 10/30 (FPLC, Pharmacia) gave the same calculation (29,000 daltons) (Fig. 2). These data indicated that the purified enzyme (GceinI) is a monomer with a relative molecular mass of 28,000 daltons.

The purified enzyme cleaved λ, Ad2, SV40, φX174 RF I and pBR322 DNAs at 5, 3, 1, 0 and 1 site, respectively. The optimum reaction conditions were at 37°C and pH 8.0 in a reaction mixture (50 μl) containing 1.0 μg λDNA, 10 mM Tris-HCl, 7 mM 2-mercaptoethanol and 7 mM MgCl2. The purified enzyme strictly required Mg2+. The magnesium ion was partly replaced by Mn2+, however, the activity of the manganese ion was only ca. 50% of Mg2+. The purified enzyme did not necessarily require monovalent cations such as Na+ and K+. The optimum NaCl concentration for the enzyme reaction was 0–150 mM.

The purified enzyme was preincubated at pH 7.5 for 5 min in the temperature range from 20 to 60°C, and then was assayed for its activity.3) The enzyme was stable up to 50°C. The purified enzyme was preincubated at 4°C for 24 hr in the pH range from 4.0 to 10.0, and then was assayed for its activity.3) The enzyme was stable between pH 5.5 and 10.0.

The purified enzyme was examined for its recognition sequence and cleavage site on DNA molecules.3) Thirteen μg of pBR322 DNA (Takara) was digested by the purified enzyme (26 units of GceinI) at 37°C for 2 hr in a reaction mixture (250 μl) containing 10 mM Tris-HCl, 7 mM 2-mercaptoethanol and 7 mM MgCl2 (pH 8.0). The resulting linear DNA molecule was dephosphorylated with alkaline phosphatase and labeled with [γ-32P]ATP using T4 polynucleotide kinase. The 5'-labeled linear DNA molecule was treated with HindIII,2) and the resultant labeled small fragment (346 bp) was sequenced by the method of Maxam and Gilbert.7) The gel pattern of the small fragment showed 5'-G-A-T-C-C-A-C-A-G- from coordinate 378 to the 3'-direction (coordinate 370 and

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Fig. 1. SDS-Polyacrylamide Gel Slab Electrophoresis of the Purified Enzyme of Gluconobacter cerinus IFO 3260. The purified enzyme (15 μg) was run and stained with 0.1% Coomassie Brilliant Blue R-250. The electrophoresis was done on a 12.5% SDS-polyacrylamide gel slab (15 x 18 x 0.2 cm), which was standardized with bovine albumin (66,000 daltons), egg albumin (45,000 daltons), trypsinogen (24,000 daltons), β-lactoglobulin (18,400 daltons) and lysozyme (14,300 daltons). Small amounts of the standard proteins flowed into the sample lane. The relative molecular mass of the purified enzyme (GceinI) was calculated to be 28,000 daltons.

Fig. 2. Relative Molecular Mass Measurement of the Purified Enzyme of Gluconobacter cerinus IFO 3260. The relative molecular mass of the purified enzyme was measured by gel filtration on a Superose 12 HR 10/30 column (1.0 x 30 cm, FPLC, Pharmacia) using a buffer containing 50 mM Tris-HCl, 7 mM 2-mercaptoethanol and 7 mM MgCl2 (pH 7.2) with a flow rate of 0.2 ml/min. Fractions of 0.5 ml were collected. The column was standardized with thyroglobulin (670,000 daltons), γ-globulin (158,000 daltons), ovalbumin (44,000 daltons), myoglobin (17,000 daltons) and vitamin B-12 (1,350 daltons). The ratios of protein void volume (Ve) minus column void volume (Vo) to column total volume (Vt) minus column void volume (Vo) were plotted against the logarithms of the relative molecular masses of the proteins. The void volume was measured by the elution of blue dextran 2,000. The relative molecular mass of the native enzyme (GceinI) was calculated to be 29,000 daltons.

*2 The restriction endonuclease HindIII cleaves pBR322 DNA around coordinate 29.
Restriction Endonuclease Gcei1

further) (data not shown). The 5'-terminal nucleotide was identified as G. These data indicated that the purified enzyme (Gcei1) recognizes the palindromic hexanucleotide sequence 5'-GGATCC-3' (recognition sequence of BamHI) and cuts between G and G.

Since the finding of the restriction endonuclease BamHI of Bacillus amyloliquefaciens H.10) a number of isoschizomers have been reported.11 However, there are only a few enzymatically detailed reports on these isoschizomers. The restriction endonuclease Gcei1 is stable against the thermal and pH-dependent treatments described above, and is produced in a large amount within the cells. The restriction endonuclease did not require monovalent cations. Unlike the restriction endonuclease BamHI, the purified enzyme expressed its full activity in the absence of NaCl or KCl. Moreover, the enzyme activity was not inhibited by the higher concentration of NaCl (150 mM). The enzyme is easier to use than the BamHI endonuclease.

It is noteworthy that the restriction endonuclease Gcei1 (28,000 daltons) described here has a monomeric structure. In contrast, the restriction endonuclease BamHI is a dimer or a tetramer of a 22,000 daltons subunit.11) And the Bst1503 endonuclease of B. stearothermophilus 1503-4R, an isoschizomer of BamHI, appears to have two active forms, a dimer (96,000 daltons) and a tetramer (180,000 daltons).12 Clarke and Hartley3) reported the presence of a loosely associated dimer of a 26,000 daltons subunit in the restriction endonuclease BstI of B. stearothermophilus NCA 1503. So the monomer-structured restriction endonuclease Gcei1 is unique among the BamHI endonuclease and its isoschizomers.

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References