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Purification and some properties of a restriction endonuclease *Ccy* I from *Clostridium cylindrosporum*

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Abstract. The restriction endonuclease *Ccy* I has been purified to homogeneity from an anaerobic bacterium, *Clostridium cylindrosporum*, by the procedures involving streptomycin sulfate precipitation, ammonium sulfate fractionation and chromatography on phosphocellulose, heparin-Sepharose 4 B and Ultrogel AcA 34. This enzyme recognizes the sequence $^{5'\text{-}GATC\text{-}3'}_{3'\text{-}CTAG\text{-}5'}$ and cleaves the phosphodiester bond at the 5′ side of G as indicated by the arrows. Several isoschizomers of *Ccy* I have been found previously which include *Sau* 3AI and *Mbo* I. Optimal NaCl concentration, pH and temperature for the enzyme to digest λDNA was 100 mM, 7.0-8.0 and 30-35°C, respectively. *Ccy* I is both acid-labile and heat-labile.

Key words: *Clostridium cylindrosporum*; Restriction endonuclease; Recognition and cleavage sequence.

Introduction

Hundreds of type II restriction endonucleases have been isolated and characterized since the first sequence-specific restriction enzyme *Hin*d II was discovered (Smith and Wilcox, 1970). The number of the documented restriction endonucleases increases each year (Roberts, 1988) mainly due to the continuous attempts to search for enzymes with new recognition and cleavage specificity. We have screened some anaerobic bacteria for restriction endonucleases and reported the restriction endonuclease *Cst* I from *Clostridium sticklandii* (Jou and Chen, 1988). *Cst* I was purified to homogeneity and shown to recognize the nucleotide sequence 5′-CTGCAG-3′ of double stranded DNA and to cleave the phosphodiester bond between A and G. The enzyme is thus an isoschizomer of *Pst* I which is widely used in molecular cloning and other researches. This paper deals with another restriction endonuclease *Ccy* I purified from *Clostridium cylindrosporum*.

Materials and Methods

Materials

The following were purchased from the sources indicated: Streptomycin sulfate, Heparin (Sigma), Phosphocellulose P-11 (Whatman), Sepharose 4B (Pharmacia), restriction endonuclease *Sau* 3AI, *Pst* I and *Hin*d III (Toyobo), *Eco*R I, Lambda, pBR 322, φX 174 and SV40 DNA (BRL), *Pvu* II, *Xmn* I and Lambda DNA (Biolabs), dGTP, dCTP, dTTP (Boehringer mannheim), α-^{32}PdATP (Amersham).

Growth of ***Clostridium cylindrosporum***

Clostridium cylindrosporum HC-1 was grown in a medium consisting of 2 g uric acid, 1.2 ml KOH (10

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N), 1.3 ml K_2HPO_4 (70%), 0.7 ml $MgSO_4$ (5%), 1.3 ml $FeSO_4$ (0.25%), 0.7 ml $CaCl_2$ (0.6%), 1 g yeast extract and 2 ml mercaptoacetic acid (80%) per liter of water according to the method of Barker and Back (1942). The pH of the medium was adjusted to 7.6-7.8 with KOH.

Cultures were grown at 35°C under anaerobic condition until the late log phase reached. The cells were harvested by centrifugation (8000 × g, 30 min) and stored at -70°C. Average yield was 0.33 g per liter.

Purification of Restriction Endonuclease

Cells were resuspended in 3 volumes (v/w) of 10 mM potassium phosphate, pH 7.4, 1 mM EDTA, 0.002% PMSF, 15 mM mercaptoacetic acid and then sonicated at 20 second intervals for 15 times while maintaining the temperature below 4°C. Cell debris was removed by centrifugation at 100,000 × g for 60 min. To remove nucleic acids from the extract, 25% streptomycin sulfate was added to the supernatant (0.2 ml of 25% streptomycin sulfate per 1 ml of supernatant) (Rubin and Modrich, 1980). The precipitate containing nucleic acids was removed by centrifugation at 12,000 × g for 30 min at 4°C. The supernatant was then brought to ammonium sulfate fractionation. The precipitate fraction of 15-70% saturation was collected by centrifugation, redissolved in and dialyzed against buffer A (10 mM potassium phosphate, pH 7.4, 1 mM EDTA, 1 mM NaN_3, 7 mM β-mercaptoethanol). The extract was then chromatographed on a phosphocellulose column (2.6 × 35 cm) which was prepared according to the method of Greene *et al.* (1978) and equilibrated with buffer A. After washing with buffer A the column was eluted with a linear gradient of 0-1 M KCl in buffer A (total volume 1 l). Fractions (5 ml) were collected and assayed for endonuclease activity. The fractions demonstrating the endonuclease activity were pooled, concentrated in dialysis sacs immersed in 40% polyethylene glycol, dialyzed against buffer A and applied to a Heparin-Sepharose 4B column (1 × 20 cm) which was prepared as described by Pirrotta and Bickle (1980). The unbound fraction was then washed with buffer A and bound fraction was eluted with a linear gradient of 0-1 M NaCl in buffer A (total volume 200 ml). Fractions of 2 ml were collected and assayed for endonuclease activity. The fractions containing endonuclease were pooled, concentrated and stored at -20°C. In order to obtain a homogeneous enzyme preparation for molecular weight determination, a portion of the purified *Ccy* I was further chromatographed on an Ultrogel AcA 34 column (1.6 × 90 cm) equilibrated with buffer A containing 0.1 M NaCl. *Ccy* I was eluted with the equilibration buffer. Fractions of 2 ml were collected, concentrated and assayed for *Ccy* I activity.

Endonuclease Assay

Endonuclease activity was assayed in a 50 μl reaction mixture containing 1 μg of λDNA, 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol and 100 mM NaCl at 37°C for 1 hour. The reaction was terminated by the addition of 10 μl of gel-loading buffer containing 100 mM EDTA, pH 8.0, 0.25% (w/v) bromophenol blue and 40% (w/v) sucrose. The digested DNA fragments were analyzed by electrophoresis on an agarose gel (1%) and stained with ethidium bromide (Maniatis *et al.* 1982). One unit of endonuclease activity was defined as the quantity of enzyme required to completely digest 1 μg of λDNA in one hour under the defined assay conditions.

Determination of the Recognition and the Cleavage Sites of ***Ccy*** *I*

The *Ccy* I and *Sau* 3AI were initially suggested as isoschizomers by their similar DNA digestion patterns on an agarose gel. To determine whether they recognize the same sequence and cleave at the same phosphodiester bond, the digestion products of a 3′ end ^{32}P-labeled DNA by *Sau* 3AI or *Ccy* I were analyzed concurrently with the base-specific cleavage products of the same DNA according to the method of Maxam and Gilbert (1980) by polyacrylamide gel electrophoresis. The 3′ end ^{32}P-labeled DNA was prepared as follows.

Five μg of pUC 19 DNA was first cut with 5 units of *Hind* III to generate a linear DNA with 3′ recessed terminus which was then filled by incubating of α-^{32}PdATP (50 μCi, ~3000 Ci/mmol) and other three unlabeled dNTPs (500 μM each) in the presence of 1 unit of Klenow fragment of DNA polymerase I. After incubation at 37°C for 15 min the reaction was terminated by the addition of 2 μl of 0.25 M EDTA and heating at 65°C for 10 min. The ^{32}P-labeled DNA was then separated from the free dNTP by running the mixture through a Sephacryl S-300 column (1 ml syringe) equilibrated with 10 mM Tris-HCl, pH 7.6, containing 1 mM EDTA. The ^{32}P-labeled DNA was

recovered by using Geneclean kit (Bio 101, Inc.) and then digested with *Xmn* I which would cleave the ^{32}P-labeled DNA into two ^{32}P-labeled fragments. The resulting labeled fragments were separated by LMT agarose gel (1%) electrophoresis and the smaller fragment (839 bp) was then recovered from the gel with the Geneclean kit. Of the ^{32}P-labeled DNA fragment in 26 μl of sterilized H_2O, 0.5 μl aliquot was used for *Ccy* I or *Sau* 3AI digestion, the other was subjected to nucleotide sequence analysis by the Maxam-Gilbert technique (1980). Both the two endonuclease digestion products and the base-specific chemical cleavage products were separated by electrophoresis on the same acrylamide gel (8%) containing 8.4 M urea.

Results and Discussion

Purification of ***Ccy I***

The purification of endonuclease *Ccy* I was summarized in Table 1. *C. cylindrosporum* cells were harvested at the late log phase at which the cells showed the highest endonuclease activity (data not shown). After streptomycin sulfate and ammonium sulfate fractionations the endonuclease activity of the extract was not yet detectable presumably due to the presence of exonucleases. The chromatography of the extract on a phosphocellulose column is shown in Fig. 1. Aliquots of the collected fractions were assayed for endonuclease activity. It was found that fractions from 56 to 135 exhibited obvious endonuclease activity. This endonuclease was designated as *Ccy* I according to the nomenclature proposed by Smith and Nathans (1973). Since it was failed to demonstrate the occurrence of another endonuclease in these fractions (fractions 56-136), the wide distribution of nuclease activity might be attributed to the prolonged incubation of the assay mixture (60 min).

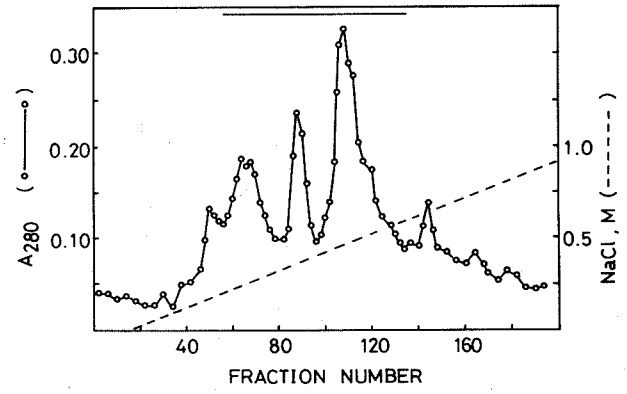

Fig. 1. Phosphocellulose chromatography of *Ccy* I. Experimental details are given under "Materials and Methods". Thirty μl aliquots of alternate fractions were assayed for endonuclease activity. The reaction mixtures (50 μl) were incubated at 37°C for 1 h. The result showed that fractions 56-135 contained *Ccy* I activity (indicated by a horizontal line).

The fractions 56 to 135 were pooled and further subjected to chromatography on a Heparin-Sepharose 4B column. The result showed that *Ccy* I was in fractions from 18 to 55 encompassing the two major protein peaks in the elution profile (Fig. 2). The fractions 18-55 were pooled and chromatographed on another Heparin-Sepharose 4B column (1.6 × 24 cm) with shallower NaCl gradient (0-0.8 M) (Fig. 3). The result showed that three protein peaks came off the column and the second peak (fractions 42-51) had the highest *Ccy* I activity (Fig. 4). The fractions 42-51 were pooled, concentrated and stored at -20°C for further experiments. Since the purified enzyme at this

Table 1. *Purification of restriction endonuclease* ***Ccy I***

Procedure	Total Protein (mg)	Total Activity (units)	Specific Activity (units/mg)	Purification Index
Crude extract	3814.7	—	—	
Streptomycin sulfate	3322.8	—	—	
Ammonium sulfate	1908.5	—	—	
Phosphocellulose	110.1	>10200	<92.6	1
Heparin-Sepharose 4 B (I)	16.7	10200	609.7	6.58
Heparin-Sepharose 4 B (II)	2.62	7000	2671.8	28.85

42.27 g of *C. cylindrosporum* was used.

stage was essentially free from exonuclease activity (data not shown), it was used for the following experiments.

Properties of ***Ccy I***

The optimal NaCl concentration, pH and temperature for *Ccy* I activity were determined with λDNA as the substrate. Optimal NaCl concentration was observed around 100 mM (Fig. 5). *Ccy* I showed 25% of its maximal activity at very low NaCl concentration.

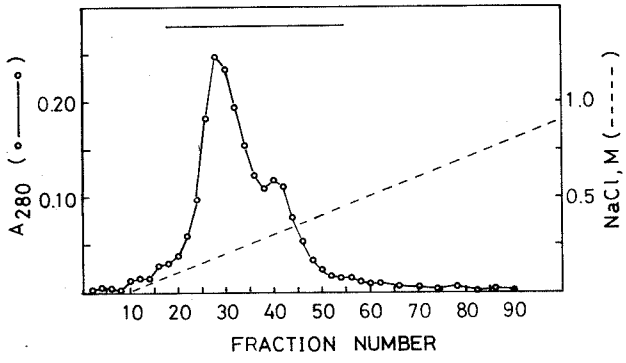

Fig. 2. Heparin-Sepharose 4 B chromatography (I) of *Ccy* I. The column (1 × 20 cm) was equilibrated with buffer A. *Ccy* I was eluted with a linear gradient of 0-1 M NaCl in buffer A (total volume 200 ml). Fractions (2 ml) were collected. Ten μl aliquots of the alternate fractions were brought to the activity assay. The total mixture (25 μl) containing 0.5 μg λDNA was incubated at 37°C for 3 h. The horizontal line indicates the fractions from 18 to 55 which showed *Ccy* I activity.

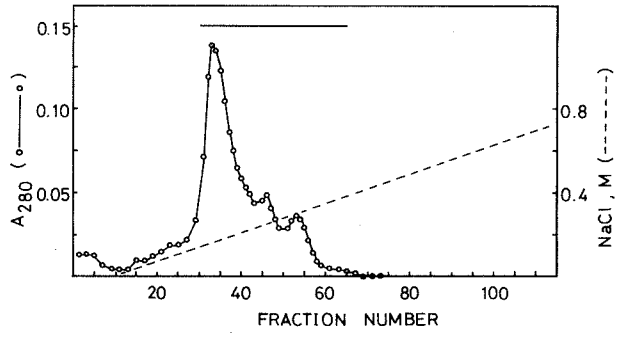

Fig. 3. Heparin-Sepharose 4 B chromatography (II) of *Ccy* I. The purified *Ccy* I (fractions 18-55 of the last column) was applied to another Heparin-Sepharose 4B column (1.6 × 20 cm). The column was eluted with a linear gradient of 0-0.8 M NaCl in buffer A (total volume 500 ml). Fractions of 4 ml were collected. *Ccy* I activity was determined as described in Fig. 2.

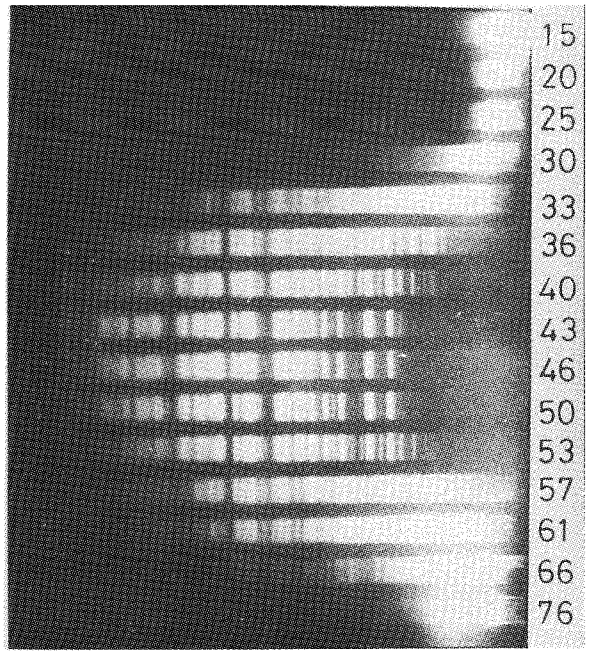

Fig. 4. Assays of the column fractions (Fig. 3) for *Ccy* I activity. Aliquots (10 μl) of the solutions in indicated fractions were incubated with 0.5 μg λDNA in 25 μl reaction mixture at 37°C for 2.5 h. After 5 μl of gel-loading buffer was added, 30 μl of the reaction mixture was loaded into an agarose gel (1%) and electrophoresed at 100 V. The gel was stained with 0.5 μg/ml of ethidium bromide and photographed under UV light.

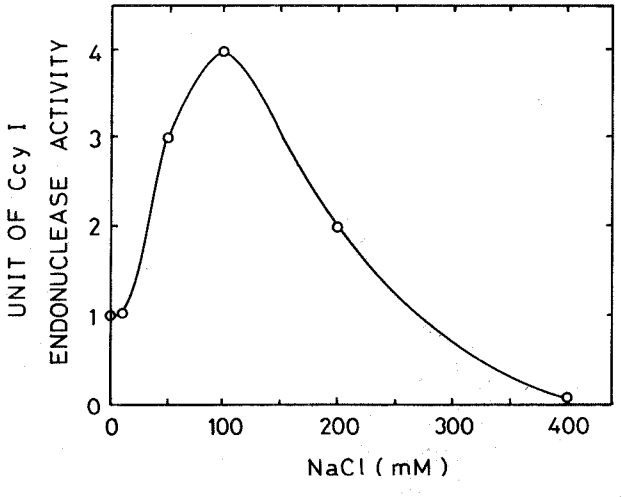

Fig. 5. Optimum NaCl concentration for *Ccy* I endonuclease activity. One μg of λDNA was hydrolyzed with 4 units of the purified *Ccy* I at indicated NaCl concentration for 1 h.

In fact, we observed *Ccy* I showing the star activity (Makula and Meagher, 1980) when the enzyme was analyzed for its recognition and cleavage specificity with NaCl concentration at 0 mM and 50 mM. The star activity however could be avoided with NaCl concentration at 100 mM (data not shown). The optimal pH between 7 and 8 for *Ccy* I activity was observed and the optimal temperature was shown between 30 and 35°C (Figs. 6 and 7). The *Ccy* I activity declined rapidly when temperature was out of this range.

The thermostability and the stability of *Ccy* I under various pH were also studied. The *Ccy* I was incubated at various temperature under the activity assay condition for 5 min and assayed for its endonuclease activity. The result showed that the enzyme was stable at temperature below 40°C, but lost its activity rapidly at temperature higher than 40°C and was completely inactivated at the temperature higher than 50°C (Fig. 8).

The effect of pH on *Ccy* I was tested by incubating *Ccy* I at 4°C under various pH conditions for 24 h and assaying the *Ccy* I activities after the samples were dialyzed against distilled water. The result showed that *Ccy* I was acid-labile, it lost its activity remark-

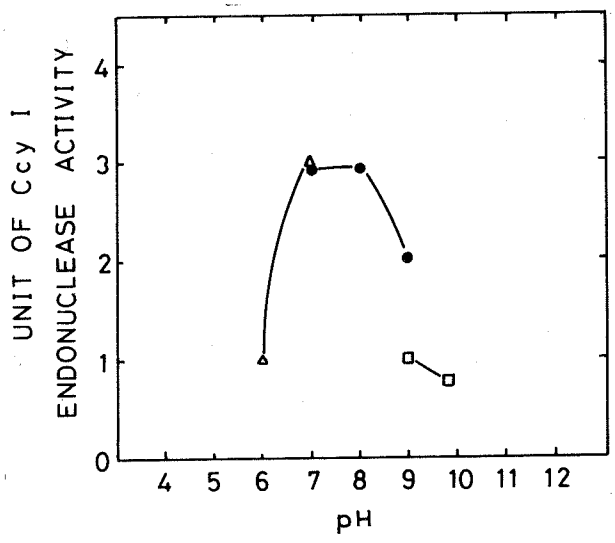

Fig. 6. Optimum pH of *Ccy* I endonuclease. One μg of λDNA was hydrolyzed with 3 units of the purified *Ccy* I at various pH for 2.5 h. pH 6.02-7.08 : Phosphate buffer (△——△). pH 7.02-9.01 : Tris-HCl buffer (●——●). pH 9.05-9.80 : Glycine-NaOH buffer (□——□)

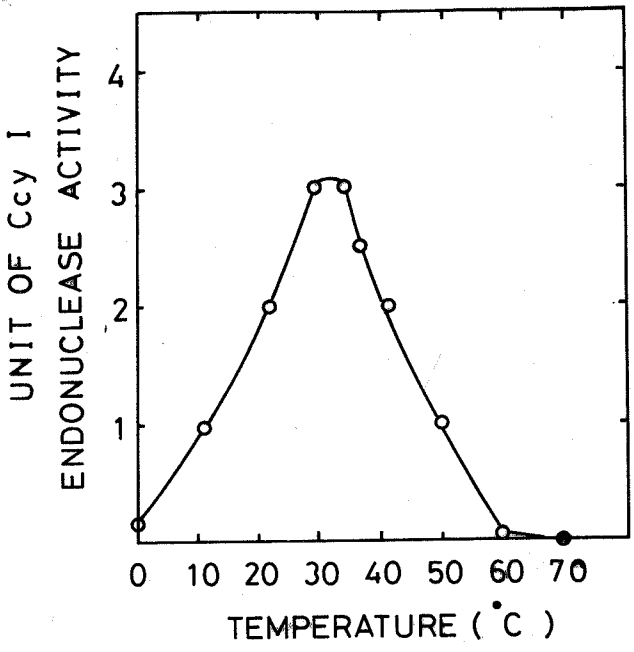

Fig. 7. Optimum temperature of *Ccy* I endonuclease. One μg of λDNA was hydrolyzed with 3 units of the purified *Ccy* I at different temperature for 2 h.

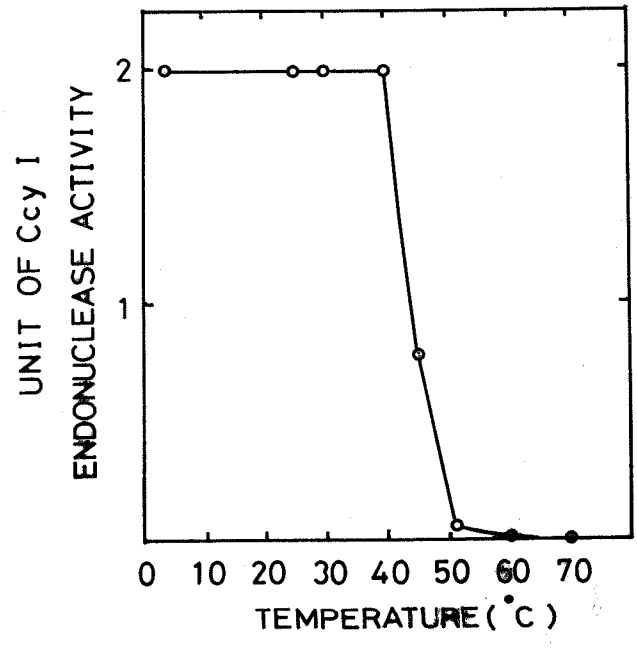

Fig. 8. Thermostability of *Ccy* I endonuclease at pH 7.4. Two units of the purified *Ccy* I was incubated at various temperatures for 5 min, cooled down immediately in ice bath and assayed for endonuclease activities under standard condition with λDNA as substrate.

ably at pH lower than 4.5 (Fig. 9). Although about 50% of *Ccy* I was inactivated at pH 9 (Fig. 6), the enzyme obviously was reactivated after the pH was brought from 9 to 7 (Fig. 9).

The molecular weight of *Ccy* I estimated by SDS-PAGE was 14,500 daltons which is close to 17,700 daltons estimated from Ultrogel AcA 34 chromatography (data not shown). When the 17,700-dalton *Ccy* I was electrophoresed on 4-30% gradient polyacrylamide gel, a single band with molecular weight around 230,000 daltons was observed (Fig. 10). It is suggested that Ultrogel AcA 34 chromatography can be used as a final purification step to obtain homogeneous *Ccy* I, and this enzyme has minimum molecular weight of about 14,500 daltons which tends to form a much larger aggregate (230,000 daltons) in the absence of reducing agent.

Determination of the Recognition and the Cleavage Specificities

The similar digestion patterns of *Ccy* I and *Sau* 3 AI on λ, Ad2, pBR322,SV40 and φX 174 DNA suggested that these two enzymes are isoschizomers with

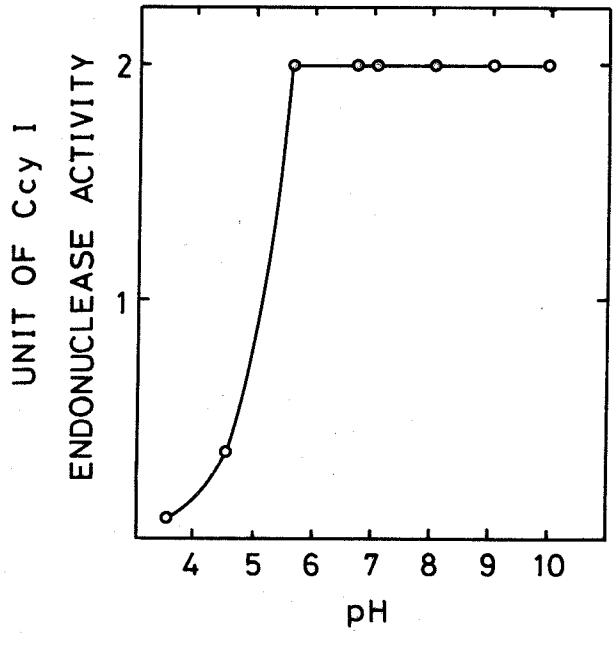

Fig. 9. Effect of pH on *Ccy* I endonuclease. Two units of the purified *Ccy* I was stored at 4°C at various pH for 24 h. After storage the endonuclease activities were assayed under the standard condition.

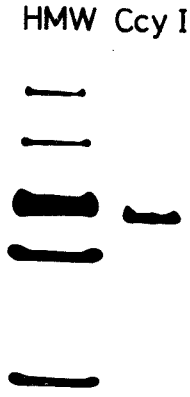

Fig. 10. Polyacrylamide gel electrophoresis of *Ccy I*. *Ccy* I (3.5 μl) was mixed with equal volume of 40% sucrose and applied on a 4-30% gradient PAGE gel (7 × 8 × 0.075 cm) (C=2.7%) in TBE buffer (90 mM Tris, 80 mM Boric acid, 2.5 mM EDTA, pH 8.4). The electrophoresis was carried out at 4°C at 420 volts for 5 h. The gel was stained by silver-staining method (Juang and Su, 1987). High molecular weight standard (HMW): Thyroglobulin (669,000), ferritin (440,000), catalase (232,000), lactate dehydrogenase (140,000), albumin (67,000).

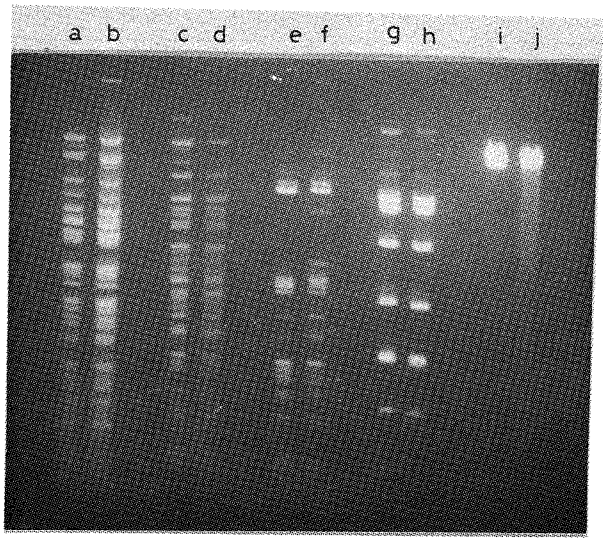

Fig. 11. Electrophoretic patterns of some DNAs cut by *Ccy* I or *Sau* 3AI. Each DNA sample (0.9-1 μg) was digested with 4 units of *Ccy* I or 8 units of *Sau* 3AI at 35°C for 2.5 h. The digestion mixtures were electrophoresed on 1% agarose gel. a : λDNA + *Sau* 3AI. b : λDNA + *Ccy* I. c : Ad2 DNA + *Sau* 3AI. d : Ad2 DNA + *Ccy* I. e : pBR 322 DNA + *Sau* 3AI. f : pBR 322 DNA + *Ccy* I. g : SV40 DNA + *Sau* 3AI. h : SV40 DNA + *Ccy* I. i : φX 174 DNA + *Sau* 3AI. j : φX 174 DNA + *Ccy* I.

identical recognition site (Fig. 11). Nucleotide sequence analysis revealed that both enzymes cleaved at the same phosphodiester bond at the 5′ end of the nucleotide G of the two fold symmetry $\overset{5'\text{-GATC-}3'}{3'\text{-CTAG-}5'}$ (Fig. 12). There are 18 restriction endonucleases so far found to have this recognition and cleavage specificity (Roberts, 1988), of which *Sau* 3AI and *Mbo* I are now commercially available.

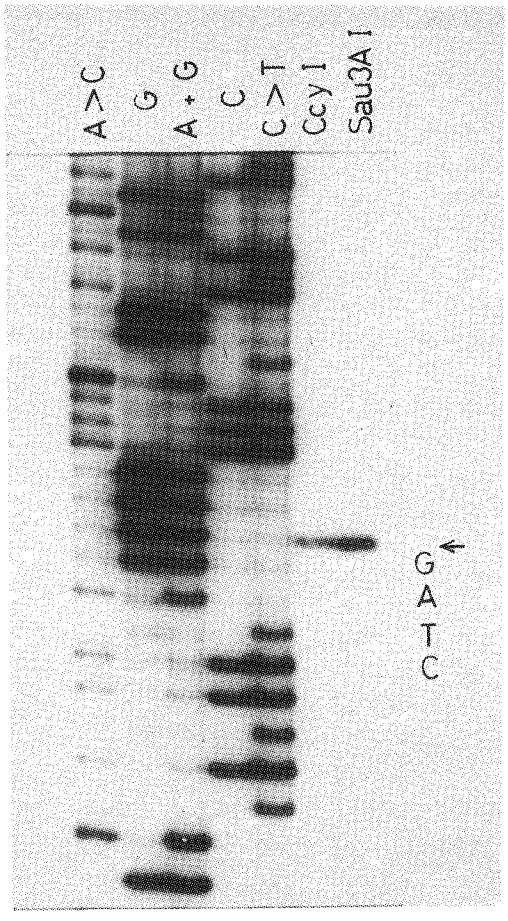

Fig. 12. Determination of cleavage site of *Ccy* I on pUC 19 DNA. The pUC 19 DNA was cut by *Hind* III, labeled with α-^{32}PdATP and further cleaved by *Xmn* I. The DNA fragment of 839 bp was isolated and used for *Ccy* I, *Sau* 3AI digestion and base-specific chemical cleavage. The digestion and cleavage products were electrophoresed on an 8% acrylamide gel at 19 mA for 2 h. The autoradiogram shows the electrophoretic patterns of the five chemical reaction mixtures as well as the digestion mixtures of *Ccy* I and *Sau* 3AI on the 839 bp fragment.

Among the endonucleases which cleave at 5′ -GATC-3′, different specificities with respect to the extent of methylation of A residues have been described (Lui *et al*., 1979). The sequence is cleaved by *Dpn* I only when it contains 6-methyl adenine and by *Mbo* I and *Dpn* II only when the adenine is not methylated. Whereas *Sau* 3AI and *Fnu* EI cleave the sequence irrespective of methylation. *Ccy* I apparently belongs to the category of *Sau* 3AI and *Fnu* EI because λDNA and pBR 322 used in the experiment shown in Fig. 11 were isolated from *dam* methylase positive strain of *E. coli* and the fragment patterns produced by digestion of these two DNA substrates with *Ccy* I and *Sau* 3AI were identical (Fig. 11). On the other hand, Ad2 DNA purchased from BRL is digestable by *dam* methylation sensitive endonuclease. Both *Ccy* I and *Sau* 3AI gave identical Ad2 DNA digestion pattern (Fig. 11). The resistance of ϕX 174 DNA to *Ccy* I and *Sau* 3AI (Fig. 11) was anticipated because the DNA does not contain the sequence 5′-GATC-3′ (Sanger, 1977).

Since an endonuclease recognizing and cleaving 5′ -GATC-3′ is a very useful tool for dissecting DNA, *Ccy* I like *Sau* 3AI will be potentially more useful than other isoschizomers which are specific for or sensitive to *dam* methylation.

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Literature Cited

Barker, H.A. and J.V. Back. 1942. *Clostridium acidi-urici* and *Clostridium cylindrosporum*, organisms fermenting uric acid and some other purines. J. Bacterial. **43:** 291-304.

Greene, P.J., H.L. Heyneker, F. Bolivar, R.L. Rodriguez, M.C. Botlach, A.A. Covarrubias, A.A. Backman, K. Russel, D.J. Tait, and H.W. Boyer. 1978. A general method for the purification of restriction enzymes. Nucleic Acids Res. **5:** 2373-2380.

Jou, T.W. and C.S. Chen. 1988. Purification and characterization of restriction endonuclease *Cst* I from *Clostridium sticklandii*. Bot. Bull. Academia Sinica **29:** 201-208.

Juang, R.H. and J.C. Su. 1987. Polyacrylamide gel electrophoresis of proteins. *In* Y.H. Tseng, S.F. Chen and C.S. Chen (eds.), Proceedings of Electrophoretic Analysis, National Science Council, Taipei, Taiwan, ROC, pp. 69-85.

Laemmli, U.K. 1970. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680-685.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193:** 265-275.

Lui, A.C.P., B.C. McBride, G.F. Voris, and M. Smith. 1979. Site specific endonuclease from *Fusobacterium nucleatum*. Nucleic Acids Res. **6:** 1-15.

Makula, R.A. and R.B. Meagher. 1980. A new restriction endonuclease from the anaerobic bacterium, *Desulfovibrio desulfuricans*. Norway. Nucleic Acids Res. **8:** 3125-3131.

Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Agarose gel electrophoresis. Molecular Cloning, Cold Spring Habor Laboratory, New York, pp. 150-161.

Margolis, J. and C.W. Wrigley. 1975. Improvement of pore gradient electrophoresis by increasing the degree of cross-linking at high acrylamide concentrations. J. Chromatogr. **106:** 204-209.

Maxam, A.M. and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavage. *In* L. Grossman and K. Moldave (eds.), Methods in Enzymology Vol. 65. Academic Press, London New York, pp. 499-560.

Pirrotta, V. and T.A. Bickle. 1980. General purification schemes for restriction endonuclease. *In* L. Grossman and K. Moldave (eds.), Methods in Enzymology, Vol. 65. Academic Press, London New York, pp. 89-95.

Roberts, R.C. 1988. Restriction enzymes and their isoschizomers. Nucleic Acids Res. **16:** r271-r313.

Rubin, R.A. and P. Modrich. 1980. Purification and properties of *Eco*RI endonuclease. *In* L. Grossman and K. Moldave (eds.), Methods in Enzymology, Vol. 65, Academic Press, London New York, pp. 96-104.

Sanger, F., G.M. Air, B.G. Barrell, N.L.Brown, A.R. Coulson, J.C. Fiddes, C.A. Hutchison III, P.M. Slocombe, and M. Smith. 1977. Nucleotide sequence of bacteriophage ϕX 174 DNA. Nature **265:** 687-695.

Smith, H.O. and D. Nathans. 1973. A suggested nomenclature for bacterial host modification and restriction system and their enzymes. J. Mol. Biol. **81:** 419-423.

Smith, H.O. and K.W. Wilcox. 1970. A restriction enzyme from *Haemophilus influenzae*. I. Purification and general properties. J. Mol. Biol. **51:** 379-391.

圓柱芽胞梭菌核酸內切酶的純化與其性質

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利用硫酸鏈黴素的沈澱，硫酸銨分劃，磷酸纖維素管柱，肝素 Sepharose 4B 親和層析及 Ultrogel AcA 34 膠體層析，從嫌氣性菌圓柱芽胞梭菌中純化出一種核酸內切限制酶，*Ccy* I。此內切酶鑑識核苷序列 $\overset{5'\text{-}GATC\text{-}3'}{3'\text{-}CTAG\text{-}5'}$，其切割之位置在G之 5′ 端的磷酸二酯鍵。此酶與已發現的 *Sau* 3AI 及 *Mbo* I 爲同形異質酶。

Ccy I 的反應最適氯化鈉濃度爲 100mM，最適 pH 在 7.0 至 8.0，最適反應溫度在 30 至 35°C，由 SDS 膠體電泳測得 *Ccy* I 之分子量爲 14,500 道爾吞。*Ccy* I 在 40°C 以上保溫 5 分鐘或在 pH 5.5 以下 4°C 貯藏一天其活性即迅速下降，顯示 *Ccy* I 爲對熱及酸不安定的酵素。