Cloning and Nucleotide Sequence of the Agel Methylese Gene from Agrobacterium gelatinovorum IAM 12617, a Marine Bacterium†

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The Agel restriction-modification system from a marine bacterium, Agrobacterium gelatinovorum IAM 12617, recognizes the nucleotide sequence ACCGGT. The gene coding for the Agel methylese (M.Agel) was cloned into Escherichia coli DH5α MCR, and the nucleotides of the gene were sequenced. The M.Agel gene coded for a protein of 429 amino acid residues (molecular mass, 47,358 daltons). The deduced amino acid sequence of M.Agel was compared with those of other methylese and showed that there are high degrees of similarity in some cysteine-5 methylese.

Key words: DNA methylase; DNA modification; Agel; restriction-modification system

Many restriction-modification systems have been found in a wide variety of microorganisms,1) and more than one hundred genes encoding the restriction-modification systems have been cloned and expressed in Escherichia coli.2,3) Agrobacterium gelatinovorum IAM 12617, a marine bacterium, produces a new restriction endonuclease, designated Agel (= R.Agel), which recognizes the palindromic hexanucleotide sequence ACCGGT, cuts between A and C, and forms a 5′-cohesive tetranucleotide extension.4,5) We have attempted cloning of the genes responsible for the Agel restriction-modification system. We here report the cloning of the Agel methylese (M.Agel) gene and the nucleotide sequence of the M.Agel gene.

Materials and Methods

Bacterial strains and plasmids. Agrobacterium gelatinovorum*† IAM 12617 was kindly supplied by Dr. Kazuhide Yamashita, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan (IAM). Escherichia coli DH5α MCR [F′, metA, thrA15 hisD4 recA1, supF44, leuB6, araD139, rpsL20, proA2, rfaD1, gtrA66, relA1], Bethesda Research Laboratories Inc., Gaithersburg, Maryland, U.S.A., and plasmids pUC18 and pUC19 were used as host and vectors, respectively, for constructing libraries and sequencing DNA. The plasmid pBR328 was used to insert a chloramphenicol (Cm) resistance gene and an Ap′ site into the plasmid pUC18 which has an ampicillin (Ap) resistance gene.

Cloning of Agel methylese gene. Agrobacterium gelatinovorum IAM 12617 was grown in a modified marine broth, as described previously.6) Chromosomal DNA from Agel gelatinovorum IAM 12617 was prepared by the procedure of Murray and Thompson7) and digested partially with SmaI. The cloning vector for the M.Agel gene was constructed as follows. The plasmid pUC18 was digested with EcoRI and the ends of the resulting linear plasmid DNA were blunted with T4 DNA polymerase. The Agel-Digested fragments (about 1300 bp) of pBR328 were ligated with the linear pUC18 DNA. The newly constructed plasmid, designated pUC18Cm, had both the Cm′-resistance gene and the Ap′ site in addition to the Ap′-resistance gene (Fig. 1). The plasmid pUC18Cm was then cleaved with BamHI, dephosphorylated, and ligated with the SmaI-digested chromosomal DNA of Agel gelatinovorum IAM 12617. The transformation of the ligation mixtures into E. coli DH5α MCR was done by the standard methods.8) The plasmid DNAs isolated from about 10,000 Ap′- and Cm′-resistant transformants were digested completely with an excess of R.Agel, and the resulting digests were used to transform E. coli DH5α MCR. The plasmids carrying the M.Agel gene from the transformants, surviving by resistance to the R.Agel digestion, were analyzed.

Assay for Agel methylese activity. The M.Agel activity in vivo was assayed as follows. The cells from individual colonies were inoculated in a small volume of culture broth (1 ml) and incubated overnight at 37°C. The plasmid and chromosomal DNAs were prepared from colonies and tested for their sensitivity to R.Agel. The DNAs (0.5 μg) were digested in a reaction mixture containing 50 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, R.Agel (5 units), and 10 mM Tris HCl (pH 7.5). The reaction mixtures were then analyzed by agarose gel electrophoresis, and the DNA modification was detected by a lack of digestion.

Nucleotide sequencing. The nucleotides were sequenced by the dideoxy chain termination method9) using deoxyadenosine 5′-α-[35S] triphosphate and a Sequenase, version 2.0, 7-deaza sequencing kit (United States Biochemical Co., Cleveland, Ohio, U.S.A.). The deletion derivatives were

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Fig. 1. Structure of pUC18Cm.
Construction of the plasmid is described in Materials and Methods. Ap′, ampicillin resistant; Cm′, chloramphenicol resistant; EcoRI091*, EcoRI091 site (blunt-end).

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1) Studies on Restriction Endonucleases of Acetic Acid Bacteria and Allied Organisms. Part XIII. For Part XII, see ref. 21.
2) To whom reprint requests should be addressed (2 J. J. Seinachn, Fukuoka, Shizuoka 426, Japan). Retired on March 31, 1995 from Shizuoka University.
3) The name of this bacterium has been validated taxonomically."
Fig. 2. Agarose Gel Electrophoresis of Digestion Products of Chromosomal DNA of Transformant Harboring Either pUC18Cm or pAGEM2.8 Digested by R. Agel. For the experimental conditions, see the text. Lane 1, chromosomal DNA of a transformant harboring pUC18Cm digested by R. Agel; lane 2, chromosomal DNA of a transformant harboring pAGEM2.8 digested by R. Agel; M, 2DNA HindIII markers.

Fig. 3. Restriction Map of pAGEM2.8. The thick lines correspond to a vector plasmid DNA, and the box corresponds to the inserted DNA fragment. The approximate coding region is indicated by oblique lines. The arrow under the box indicates the direction of the M. Agel gene. Plac, lac promoter.

Fig. 4. Nucleotide Sequence of Agel Methylase Gene. The translation for the Agel methylase gene is shown under the DNA sequence. Putative −35 and −10 regions are underlined. A putative Shine-Dalgarno sequence is indicated by the four asterisks and a stop codon is by the one asterisk.

obtained using a kilo-sequence deletion kit (Takara Shuzo Co., Kyoto, Japan).

Nucleotide sequence accession number. The nucleotide sequence data reported here will appear in the DDBJ Nucleotide Sequence Database under the accession number D78259.

Enzymes and chemicals. All the restriction endonucleases used in this experiment, e.g., Sau3AI, Agel, BamHI, Accl, and EcoRI101I, and T4

\[ \text{Plac} \]

\[ \text{BamHI} \text{ApaI} \text{Accl} \text{SacI} \text{ApaI} \]

\[ \text{pUC18CM} \]

\[ \text{M.Agel} \]

\[ \text{pAGEM2.8} \]
DNA polymerase. T4 DNA ligase, and alkaline phosphatase were obtained from Nippon Gene Co., Tokyo, Japan. Deoxyadenosine 5'-ni(3')-triphosphate was from ICN Radiochemicals, Irvine, California, U.S.A.

Results
Cloning of Age1 methylase gene

The plasmid clones carrying the M.Age1 gene were isolated by selecting the recombinants which protectively modified themselves against digestion by R.Age1. One plasmid clone was selected by this method. The recombinant plasmid, designated pAGEM2.8, contained an insert of approximately 2.8 kb. The chromosomal DNA of the transformant carrying this plasmid displayed complete resistance to R.Age1 (Fig. 2). The M.Age1 gene expression was dependent on the orientation of the inserted DNA in the vector. These results suggested that the transcription was initiated from a vector-born promoter. In the deletion experiment using the plasmid pAGEM2.8, the M.Age1 gene was found to be located in the 1.7 kb fragment (Fig. 3).

Fig. 5. Comparison of Age1 Methylase with Cytosin-5 Methylases.

The amino acid sequence of the five methylases was aligned by the program Clustal W. 29 The conserved regions (Regions 1 through XI) were indicated by doted lines on the amino acid sequences. The asterisks and dots under the sequences represent identical and similar amino acids, respectively.
Nucleotide sequence of \textit{AgrE} methylase gene

The nucleotides of the 1.7-kb fragmen were sequenced. An open reading frame was found in this fragment (Fig. 4). The first methionine codon was at position 227 in the open reading frame, and the frame continued until a TAG terminator at position 1514. The open reading frame, in positions 227 through 1513, 1287 bp, predicted a protein containing 429 amino acids with a calculated molecular mass of 47,358 daltons. A putative Shine-Dalgarno sequence (GGGA) was found four bp upstream from the ATG codon. Putative promoter-like structures were observed at –10 (TATATT, positions 133 to 138) and –35 (TGTTCT, positions 110 to 115) regions.

\textbf{Comparison of \textit{AgrE} methylase with other methylases}

The deduced amino acid sequence of \textit{M.AgrE} was compared with those of other methylases from the protein databases, Swiss-Prot and PIR, by the method of Smith and Waterman. As shown in Fig. 5, the \textit{AgrE} methylase from \textit{A. gelatinovorum} IAM 12617 had significant homologies with cytosome-5 methylases. The alignments of the deduced amino acid sequences of \textit{M.AgrE} showed the ten conserved regions (Regions I through X) which are characteristic of cytosome-5 methylases. In addition to the ten conserved regions mentioned above, a moderately conserved region was found (Region XI). The results obtained here suggested that the \textit{AgrE} methylase is classified as a cytosome-5 methylase.

\textbf{Discussion}

Since our findings of a new restriction endonuclease, \textit{AgrE}, which recognizes ACCGGT and cuts between A and C, in \textit{A. gelatinovorum} IAM 12617, the only isochizomer, \textit{PinAI}, had been reported in \textit{Pseudomonas inequalis}. However, the \textit{PinAI} restriction-modification system has not been analyzed.

The newly constructed plasmid pAGEM2.8 was found to express \textit{M.AgrE}, since complete protection was shown against the \textit{R.AgrE} digestion of chromosomal DNA isolated from the cells bearing this plasmid. In this study, the only methylase gene in the \textit{AgrE} restriction-modification system of \textit{A. gelatinovorum} IAM 12617 was cloned in a single step. However, the \textit{R.AgrE} gene has not been cloned. We have analyzed nucleotide sequences in either the upstream or the downstream (about 1 kb in both) of the \textit{M.AgrE} gene, however, no open reading frame of the \textit{R.AgrE} gene has been found. Generally, the methylase and the restriction endonuclease genes lie adjacent to one another in restriction-modification systems. The \textit{AgrE} restriction-modification system described here is assumed to be exceptional.

Some of the genes of the methylases that recognize nucleotide sequences, viz., CCGG and CGCCGC, similar to \textit{M.AgrE} (ACCGGT) have been sequenced, and the deduced amino acid sequences have been reported. Among the cytosome-5 methylases that recognize CCGG, e.g., \textit{M.BsaFl}, \textit{M.Hpyll}, \textit{M.MspI}, did not show high degrees of similarity to \textit{M.AgrE} reported here in the deduced amino acid sequences. In contrast, \textit{M.AgrE} was highly similar to some methylases, e.g., \textit{M.DdeI} (CTNAG), \textit{M.NgoMlI} (GGCC), \textit{M.YoxI} (GATCG), and \textit{M.FnuDI} (GGCC) (Fig. 4). We can also conclude that there appear not to be remarkable relationships necessarily between recognition sequences on DNA and deduced amino acid sequences of protein in cytosome-5 methylases.

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\textbf{References}