HOKKAIDO UNIVERSITY

Title	NUCLEOTIDE SEQUENCE OF THE CAPSID PROTEIN GENE OF BEAN YELLOW MOSAIC VIRUS CHLOROTIC SPOT STRAIN
Author(s)	TAKAHASHI, Tohru; UYEDA, Ichiro; OHSHIMA, Kazusato; SHIKATA, Eishiro
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 64(2), 152-163
Issue Date	1990-01
Doc URL	http://hdl.handle.net/2115/13092
Type	bulletin (article)
File Information	64(2)_p152-163.pdf

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

J. Fac. Agr. Hokkaido Univ., Vol. 64, Pt. 2 : 152~163 (1990)

NUCLEOTIDE SEQUENCE OF THE CAPSID PROTEIN GENE OF BEAN YELLOW MOSAIC VIRUS CHLOROTIC SPOT STRAIN

Tohru TAKAHASHI, Ichiro UYEDA
Kazusato OHSHIMA and Eishiro SHIKATA
Department of Botany, Faculty of Agriculture,
Hokkaido University, Sapporo 060, Japan

Received Octover 31, 1989

Introduction

The potyviruses including bean yellow mosaic virus (BYMV) have a flexuous thread particle of about 750 nm in lengths.[21] The virion contains a single component of (+)sense single-stranded RNA genome of approximately 10K nucleotides.[3,4,6,27] These RNAs are thought to be covalently linked to a small protein (VPg)[16,33] at the 5'terminal nucleotide and have a polyadenylated track at the 3'terminus.[16,18]

So far, seven virus-encoded polypeptides were found to be associated with potyviral infections : the coat protein (CP), helper component (HC), VPg, cylindrical inclusion (CI), cell to cell transfor function protein (28-34K) and two nuclear inclusion proteins (NIa and NIb). The cistrons for each of these genes have been mapped to the potyviral genomes.[3,5,6,9,18,27] Genomes of potyviruses are expressed through the production of a large polyprotein which is proteolytically cleaved in a similar manner to that of picorna-, toga- and comoviruses.[11,17,40,45]

These studies together with the sequence analyses of the 3' terminal region of some potyviruses revealed that the predicted coat protein cistron was located upstream from the polyadenylated 3' terminus of the genome.[1,2,3,6,8,12,14,25,27,41]

Amino acid sequence of the coat protein is an increasingly important taxonomic criterium of the potyvirus as the sequence informations are accumulating and comparison of major potyviruses and their strains become available. It has been pointed out that N-terminus of the amino acid sequence is specific for each potyviruses.[20,36,37,38]

In this paper, we report the nucleotide sequence of the 3' terminal region of the BYMV-CS. Coat protein gene is mapped in the deduced sequence and its amino acid sequence was determined.

Materials and Methods

Virus and Viral RNA extraction. BYMV-CS was originally isolated from

red clover[23] and maintaind in broad bean (*Vicia fava* cv. Wase-Soramame). The virion was purified from infected broad bean leaves by a procedure described previously.[39] Genomic RNA from purified BYMV-CS was prepared by a method similar to that described previously.[4,7,17] To a 2 mg/400 μl suspension of the virus was added an equal volume of 200 mM ammonium carbonate (pH 9.0) containing 2 mM EDTA, 2% SDS, 200 μg/ml bentonite and 10 μg/ml Proteinase K. After incubation for 20 min at room temperature, the mixture was layered (1 ml/tube) onto a liner-log 7.5%-33.4% (w/v) sucrose gradient containing 150 mM NaCl, 15 mM sodium citrate (pH 7.0). Gradients were centrifuged for 9 hr at 24,000 rpm in a Hitachi RPS-27 rotor. Viral RNA, which sedimented as a single sharp zone, was recovered by precipitation with 2.5 volumes of ethanol, and used for cDNA synthesis.

Complementary DNA synthesis. The first strand of complementary DNA was synthesized[13] in a reaction mixture containing 50 mM tris-HCl (pH 7.9 at 42°C), 10 mM $MgCl_2$, 30 mM KCl, 1 mM DTT, 1 mM each of dATP, dCTP, dGTP, dTTP, 80 μg/ml of oligo (dT_{12-18}), 50 μg/ml actinomycin D, 100 μCi/ml of [α-^{32}P] dCTP, 1600 U/ml reverse transcriptase (BRL) and 200 μg/ml of viral RNA and incubated at 42°C for 40 min. After phenol-chloroform extraction and ethanol precipitation, the first strand cDNA was converted to the double stranded cDNA in a reaction mixture containing 100 μCi/ml [^{3}H] dCTP, 20 mM tris-HCl (pH 7.4), 5 mM $MgCl_2$, 10 mM ammonium sulfate, 100 mM KCl, 15 μM β-NAD, 50 μg/ml BSA, 10 μM dNTPs, 4.3 U/ml RNase H (Takara shuzo Co.), 115 U/ml *E. coli* DNA polymerase I (Takara shuzo Co.), 6 U/ml *E. coli* DNA ligase (BRL). The mixture was incubated sequentially for 1 hr at 12°C and for 1 hr at 22°C. In a second experiment, cDNA was synthesized using a cDNA Synthesis System Plus purchased from Amarsham Corp. After chromatography on Sephadex G-50, the double stranded cDNA was tailed with dCTP using terminal deoxynucleotidyl transferase (Takara shuzo Co.) and annealed with *Pst* I cut dG-tailed pBR 322 (BRL) and recombinant cDNAs were used to transform *Escherichia coli* HB 101 cells made competent by $CaCl_2$.[28]

Southern blot hybridization. Recombinant DNAs were screened for cDNA inserts by a southern blot hybridization.[38] Viral RNA in 5 mM $MgCl_2$ and 25 mM glycine-NaOH (pH 9.0), was incubated at 37°C for 3 hr[32], and precipitated by ethanol. Magnesium cleaved viral RNA was labeled using [γ-^{32}P] ATP and T4 polynucleotide kinase (Takara shuzo Co.) and used as a probe.

cDNA sequence determination. Insert cDNAs digented with *Pst I* were ligated into the polylinker region of the replicative form of the bacteriophage M13mp19 and it was used to transform *E. coli* JM109 made competent by $RuCl_2$.[15,19] An ordered set of deleted clones was made by exonuclease-III attacking method[19] and sequenced by dideoxy chain termination method using a sequencing kit (Takara shuzo Co.).

Sequences were compiled and analysed using a NEC 9801 XL computer and a software programmed by GENETYX (SDC Software Development Co.).

Characterization and amino acid sequencing of the capsid protein. Immediately after the purification, 1 mg of purified virus was resuspended in 2 ml of 50 mM sodium borate buffer at pH 8.0, and incubated with 2 μg of trypsin (Sequence grade, Boehringer Mannheim) for 30 min at room temperature.[2,36] The trypsin-resistant particles which contain core subunits was collected by centrifugation at 40,000 rpm for 2 hr at 4°C in Hitachi RP-65 rotor. The final pellet was resuspended in double distilled water. SDS-PAGE of BYMV-CS intact or core capsid proteins was carried out in 12.5% polyacrylamide gel electrophoresis system of LAEMMLI.[24] Amino acid composition of BYMV-CS intact coat protein was determined by using hydrolysate treated in 6N HCl at 110°C for 24 hr, and the amino acid analysis was performed by a Hitachi-835 analyzer.[26] The N-terminal sequence of intact or trypsin treated virion were performed by a sequential Edman degradation method in 477A Protein Sequencer and 120A PTH Analyzer (Applied Biosystems).

Results

Recombinant DNA Molecules Containing BYMV-CS

About four hundreds of recombinant DNA plasmids containing cDNA interts derived from BYMV-CS RNA were identified. The cDNA inserts of those plasmids ranged in size from 500 to 2,200 base pairs (bp), and were analyzed by

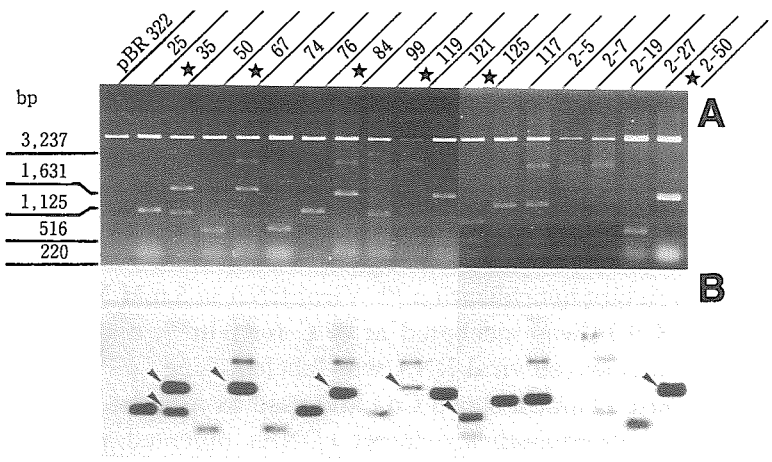

Fig. 1. Restriction analysis and southern blottds of DNA from the clones. Plasmid DNAs containing cDNA inserts were restricted with *Pst*I and electrophoresised in 1.5% agarose gel(A). DNAs were transferred onto nitrocellulose, hybridized with ^{32}P labeled genomic RNA and autoradiographed(B).

Southern blot hybridization with 5' ^{32}P-labeld genomic RNA probe as shown in Fig. 1. In this study, we selected 6 recombinant cDNA clones which covered 3' terminal portion (pCS35, pCS67, pCS84, pCS119, pCS125, pCS2-50,) of the genome RNA. The relationships between the cDNA inserts and the BYMV-CS genomic RNA are presented in Fig. 2, along with a strategy used to determine the nucleotide sequence (Fig. 3). Since these clones did not contain the 3' terminus as they lacked the poly A sequence up stream of the oligo dC tail, the second cDNA synthesis was performed using a kit purchased from Amarsham Corp. The plasmid pCS201 was found to contain the poly A sequence.

DNA Sequencing

The 1,392 nucleotides sequence determined from pCS119 and pCS201 was presented in Fig. 4. The DNA sequence contains one large open reading frame of 1,218 nucleotides which is terminated by a single TAG codon (Fig. 4.). The

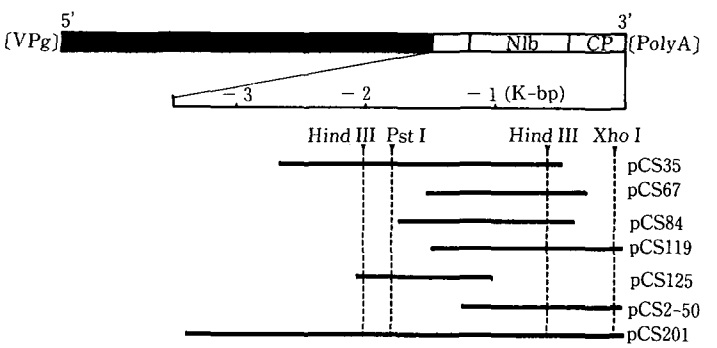

Fig. 2. The physical maps and asignment of complementary DNA recombinant molecules to BYMV-CS genomic RNA.

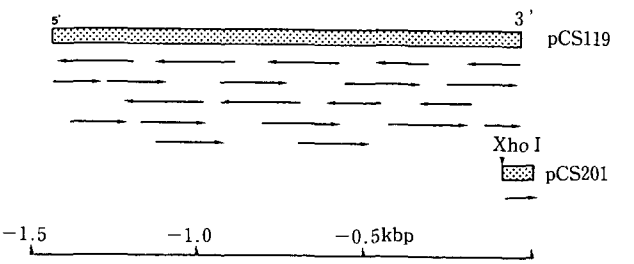

Fig. 3. The nucleotide sequencing strategy of 3'terminal region of BYMV-CS. The horizontal arrows indicated the direction and extent of sequence determination. Since pCS119 did not contained poly A sequence, poly A and upstream sequences were determined using a fragment of pCS201 digested with *Pst*I and *Xho*I.

```
-406  Lys Glu Lys Gly Asp Leu Trp Phe Met Ser His Arg Gly Ile Gln Val Asn Gly Met Trp
-1392 AAA GAG AAG GGT GAC TTG TGG TTT ATG TCA CAT AGA GGG ATA CAA GTG AAT GGT ATG TGG

-386  Ile Pro Lys Leu Glu Glu Glu Arg Ile Val Ser Ile Leu Glu Trp Asp Arg Ala Leu Gln
-1332 ATT CCA AAG CTG GAG GAA GAG CGA ATT GTA TCA ATT CTT GAA TGG GAT AGA GCC CTC CAA

-366  Pro Glu His Arg Leu Glu Ala Ile Cys Ala Ser Met Ile Glu Ala Trp Gly Tyr Pro Glu
-1272 CCA GAA CAC AGG CTT GAA GCA ATT TGT GCA TCC ATG ATA GAA GCA TGG GGT TAC CCT GAA

-346  Leu Leu Asn His Ile Arg Lys Phe Tyr Leu Trp Val Leu Gly Gln Ala Pro Tyr Ser Gln
-1212 TTA CTG AAC CAT ATC CGT AAA TTC TAC CTC TGG GTC TTG GGT CAG GCA CCA TAC AGT CAA

-326  Leu Ser Ala Glu Gly Lys Ala Pro Tyr Ile Ser Glu Val Ala Leu Lys His Leu Tyr Thr
-1152 CTC AGT GCT GAA GGA AAA GCA CCA TAC ATA TCA GAG GTT GCC CTC AAG CAT TTG TAC ACA

-306  Glu Glu Lys Ile Thr Pro Ala Glu Leu Glu Arg Tyr Asn Val Ala Leu Val Asp Cys Ala
-1092 GAA GAA AAG ATC ACA CCG GCA GAG CTT GAA AGG TAC AAC GTA GCC CTA GTT GAT TGT GCG

-286  Glu Pro Glu Glu Asp Glu Val Ile Leu Cys Cys Phe Gln Ser Asp Gln Glu Lys Leu Asn
-1032 GAG CCA GAA GAA GAT GAA GTA ATT CTG TGC TGT TTC CAA TCA GAC CAA GAG AAA CTC AAT

-266  Ala Ser Glu Lys Lys Lys Asp Lys Asp Lys Lys Val Glu Asp Gln Ser Thr Lys Glu Ser
-972  GCT AGT GAA AAG AAG AAA GAT AAG GAC AAG AAG GTT GAA GAT CAA TCA ACC AAA GAA AGT

-246  Glu Gly Gln Ser Ser Lys Gln Ile Ile Pro Asp Arg Asp Val Asn Ala Gly Thr Thr Gly
-912  GAG GGG CAG AGT AGC AAA CAA ATC ATA CCT GAT CGG GAC GTG AAT GCA GGA ACA ACT GGA

-226  Thr Phe Ser Val Pro Arg Leu Lys Lys Ile Ala Gly Lys Leu His Ile Pro Lys Val Asn
-852  ACT TTC TCA GTC CCA AGA CTC AAG AAG ATA GCA GGA GGG CTC CAC ATT CCG AAG GTG AAT

-206  Gly Lys Ile Val Leu Asn Leu Asp His Leu Leu Glu Tyr Asn Pro Ser Gln Asp Asp Ile
-792  GGA AAG ATT GTA CTA AAC CTG GAC CAC TTG TTG GAG TAC AAT CCG TCA CAG GAT GAT ATC

-186  Ser Asn Thr Ile Ala Thr Asp Glu Gln Phe Lys Ala Trp Tyr Asn Gly Val Lys Gln Ala
-732  TCA AAT ACC ATT GCA ACT GAT GAA CAA TTT AAA GCA TGG TAC AAT GGA GTG AAG CAG GCT

-166  Tyr Glu Val Glu Asp Ser Gln Met Ser Ile Ile Leu Asn Gly Leu Met Val Trp Cys Ile
-672  TAT GAA GTT GAA GAT TCA CAG ATG AGC ATT ATT TTG AAT GGG TTG ATG GTA TGG TGT ATC

-146  Glu Asn Gly Thr Ser Gly Asp Leu Gln Gly Glu Trp Thr Met Met Asp Gly Asp Glu Gln
-612  GAA AAT GGA ACA TCA GGT GAC TTA CAA GGT GAG TGG ACC ATG ATG GAT GGT GAT GAA CAA

-126  Val Thr Tyr Pro Leu Lys Pro Ile Leu Asp Asn Ala Lys Pro Thr Phe Arg Gln Ile Met
-552  GTG ACA TAC CCC CTG AAA CCC ATC TTA GAT AAT GCA AAG CCA ACA TTT CGC CAG ATA ATG

-106  Ser His Phe Ser Gln Val Ala Glu Ala Tyr Ile Glu Lys Arg Asn Ala Thr Glu Arg Tyr
-492  TCG CAC TTT TCA CAG GTT GCT GAA GCT TCT ATA GAG AAG AGG AAT GCA ACT GAG AGG TAT

 -86  Met Pro Arg Tyr Gly Leu Gln Arg Asn Leu Thr Asp Thr Gly Leu Ala Arg Tyr Ala Phe
-432  ATG CCG CGT TAT GGC CTC CAG AGG AAC TTA ACT GAC TAT GGT TTG GCT AGA TGT GCT TTT

 -66  Asp Phe Tyr Arg Leu Thr Ser Arg Thr Pro Val Arg Ala Arg Glu Ala His Met Gln Met
-372  GAT TTC TAC AGG CTA ACT TCG AGA ACT CCT GTG CGT GCT AGG GAA GCA CAT ATG CAG ATG

 -46  Lys Ala Ala Ala Ile Arg Gly Lys Ser Ans Arg Leu Phe Gly Leu Asp Gly Asn Val Gly
-312  AAG GCA GCA GCA ATT AGA GGC AAG TCA AAC CGA TTA TTT GGT CTT GAT GGA AAT GTT GGA

 -26  Thr Asp Glu Glu Asn Thr Glu Arg His Thr Ala Gly Asp Val Asn Arg Asp Met His Thr
-252  ACA GAC GAG GAG AAC ACA GAA AGA CAC ACA GCA GGA GAT GTC AAT CGT GAT ATG CAC ACC

  -6  Met Leu Gly Val Arg Ile ***
-192  ATG CTT GGT GTT CGT ATT TAG A GTATCCGTCT ATAAATTCTC TGAAATTTGG CGTTACATTA CTTAA

-125  TACTA TGTATTAGCG AGGTTTTACC TCCAGCATTT TAAATTCAGT AAGTGTTTCA TTCTCTCTAC TCTGACA

 -53  GGG TAAGCTGTTA GTGAGGTTTC CTCGAGTGGG CCTGATCTTT GTAGAGCGAC PolyA
```

Fig. 4. The cDNA sequence of BYMV-CS as the genomic RNA sense. The predicteded amino acid sequence of the large open reading frame is presented in the standard three letter code above the nucleotide sequence. The cleavage site is indicated by black arrow.

polypeptide encoded for is 406 residues long (Fig. 4.). The other reading frames contain numerous stop codons.

Coat protein analysis

BYMV-CS coat protein has a molecular weight of about 30,808 which was estimated from amino acid composition analysis (Table 1). This value is in good agreement with that estimated from SDS-PAGE analysis (Fig. 5). An attempt to

TABLE 1. Amino Acid Composition of the BYMV-CS Coat Protein.

Amino acid residue	BYMV-CS Predicted NA Seq.	BYMV-CS Determined chemically
Met	10	9
Asx	37	36
Thr	18	17
Ser	16	15
Glx	33	34
Gly	19	21
Ala	18	19
Cys	1	1
Val	14	14
Ile	15	13
Leu	19	20
Tyr	10	10
Phe	7	7
Lys	21	21
His	6	6
Arg	17	17
Pro	9	10
Trp	3	N
mol. wt.	30,998	30,808

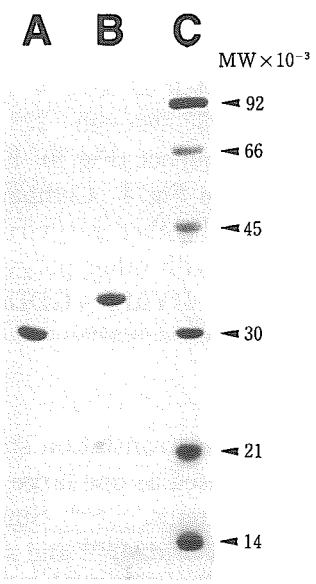

Fig. 5. Polyacrylamide gel electrophoresis of intact (B) and trypsin treated (A) coat protein of BYMV-CS. Numbers on the right are Mw of marker proteins (C)

determine the amino acid sequence of the N-terminal portion of purified BYMV-CS coat protein was unsuccessful, presumably due to a blocked N-terminus. Similar situations are also encountered with those of TEV-HAT, TVMV and PVY.[2,6,35]

But amino acid sequence of these viruses were obtained after removing the N-terminal about 20 to 30 amino acid residues by partial digestion with trypsin. When purified BYMV-CS was likewise treated with trypsin, the intact 32K coat protein resulted in the generation of a "trypsin resistant core" with an estimated molecular weight of 29K as determined by SDS-PAGE (Fig. 5). The sequence of

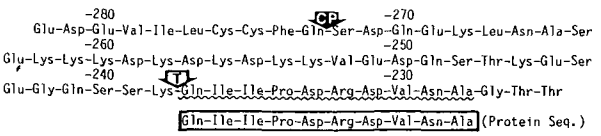

Fig. 6. Comparison between the N-terminal portion of the amino acid sequence predicted from the nucleotide sequence and that determined by a direct chemical method. The amino acid sequence of the core casid protein determied by the direct chemical method was boxed.

the N-terminal portion of this trypsin-resistant core was determined chemically to be Q-I-I-P-D-R-T-V-N-A. This sequence was identical to amino acid residues -237 through -228 from the carboxyl terminus predicted from the nucleotide sequence (Fig. 6). From these results, the coat protein gene was identified as shown in Fig. 4. Comparison of the amino acid sequence of BYMV-CS coat protein with other potyviruses showed that the extents of amino acid sequence homology with other potyvirus coat proteins are 92%, 64%, 53%,59%, 59% and 53% for BYMV-GDD, TEV, TVMV, PeMV, PVY and SCMV, respectively.[3,6,8,12,14,34]

Discussion

Cell free translation studies of some potyviruses indicated that the genome was generated as one large protein which was subsequently processed to give the mature proteins.[5,7,18,40] Simmilar gene expression mechanism was demonstrated for picornaviridae, [22] and subsequently been found for cowpea mosaic virus[42] or other plant viruses.[43] It was shown that the enzymes involved were cystein like proteinase and catalysis occured on the carbonyl side of a glutaminyl residue. In potyviruses, the protein cleavages occured on Gln-Gly, Gln-Ser or Gln-Ala[1,2,3,6,8,12,27,31], and amino acids with "bulky" side chains are found adjacent to the glutamine residue and these amino acids are tyrosine, phenylalanine or histidine of aromatic amino acids.[1] In our amino acid sequence predicted from the nucleotide sequence, these glutamine-X dipeptide were found at five positions. The molecular weight of the BYMV-CS protein was estimated to be 32K by polyacrylamide gel electrophoresis. The value suggests that the probable cleavage site is between the glutamine and serine residues found at amino acid position -273 to -272. This would result in a 30,998 molecular weight capsid protein molecule, which is in good agreement with the molecular size of 30,808 estimated by amino acid composition analysis and also 32K estimated by polyacrylamide gel electrophoresis. In addition, chemical sequencing of the protein digested with trypsin yielded amino terminal protein of ten amino acids which was identical to amino acid residues -237 to -228 of that deduced from the nucleotide sequence. This ten amino acid sequence was not found at any other locations in the putative

polyprotein. Tryptic cleavage after the lysine residue at -238 of the predicted coat protein would yield a trypsin resistant core protein with molecular weight of 27,479 similar to that estimated from polyacrylamide gel electrophoresis. Thus, we concluded that coat protein is produced by proteolytic cleavage of BYMV-CS polyprotein between the glutaminyl reridue at amino acid position -274 and the following seryl residue. The same dipeptide was apparently cleaved in TEV -HAT and TVMV. Alignment of amino acid sequences showed that BYMV-CS coat protein has significant homologies with other potyvirus coat proteins. The inner portion of amino acid sequences have higher homologies than that of N- and C-terminal sequences between different viruses (Fig. 7). Comparison of the amino acid sequence between BYMV-CS and BYMV-GDD showed that extensive homology existed even in N- and C-terminal regions. These results were also consistent with the notion that immunological specificities of potyviruses were located in N- and C-terminal arms arranged on the surface of virus particle as proposed previously.[20,36,37,38].

Nucleotide sequence of the 3'noncoding sequence of BYMV-CS has no homology to that of other potyviruses sequenced to data and no polyadenylation signals were found. However significant homology in the 3'noncoding region between BYMV-CS and BYMV-GDD[14]. was found.

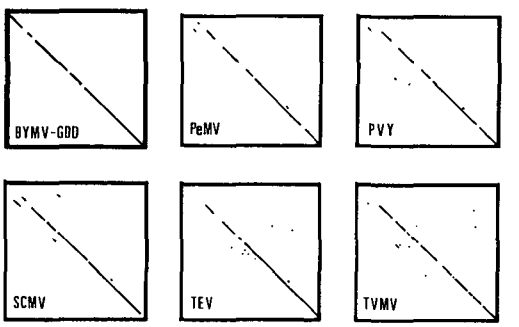

Fig. 7. Harr Plot analysis of six potyvirus(vertical) coat proteins with BYMV -CS(horizontal). The dot-plots were made using a window of 10 and stringency of 0.7.

Summary

The sequence of 1,392 nucleotides of the 3'region of the bean yellow mosaic virus chlorotic spot strain (BYMV-CS) RNA has been determined. There is a single open reading frame (ORF), from the 5'end, of 1,218 nucleotides upstream from a 173 nucleotides non-coding region that is polyadenylated. The ORF encodes a polypeptide of 406 amino acids. The cistron of the genome encoding a

potyviral capsid protein is adjacent to the 3'non-coading region. The chemically determined amino acid composition of the coat protein was similar to that predicted from the nucleotide sequence. The sequence of the 10 amino terminal amino acids of the BYMV-CS coat protein was determined chemically after removing blocked amino terminal oligo-peptides. Our data suggeste that the BYMV-CS coat protein, like that of other potyviruses, is a product of the maturation of a large polyprotein. The putative cleavage site is at a glutamine -serin dipeptide. The capsid protein gene consists of 819 nucleotides and corresponds to a region coding 273 amino acids which have a calculated molecular weight (Mw.) of 30,998. The amino acid sequence of BYMV-CS coat protein is 53 to 64% homologous to other potyvirus coat protiens and most of the variations are found in the amino terminal region. And it was 92% identical to coat protein gene of BYMV-GDD.

Key words: BYMV, Capsid protein, Nucleotide Sequence.

Acknowledgment

This work was supported in part by a Grant in Aid for Scientiffic Reseach (No. 63790330) from the Ministry of Education, Science and Culture, Japan and Legume Association (Mame-Rui Kikin Kyokai) and a grant from Akiyama Foundation.

The authors thank Dr. K. Fujinaga, Sapporo Medical College, for his advice on cDNA cloning and sequencing techniques.

Literature Cited

1) ALLISON, R. F., SORENSON, J. C., KELLY, M. E., ARMSTRONG, F. B. and DOUGHERTY, W. G.: Sequence determination of the capsid protein gene and flanking region of tobacco etch virus: Evidence for synthesis and processing of a polyprotein in potyvirus genome expression. *Proc. Natl. Acad. Sci. USA* **82**: 3969-3972. 1985
2) ALLISON, R. F., DOUGHERTY, W. G., PARKS, T. D., WILLIS, L., JOHNSTON, R. E., KELLY, M. and ARMSTRONG, F. B.: Biochemical analysis of the capsid protein gene and capsid protein of tobacco etch virus: N-terminal amino acids are located on the virion's surface. *Virology* **147**: 309-316. 1985
3) ALLISON, R., JOHNSTON, R. E. and DOUGHERTY, W. G.: The nucleotide sequence of the coading region of tobacco etch virus genomic RNA: Evidence for the synthesis of a single polyprotein. *Virology* **154**: 9-20. 1986
4) BRAKKE, M. K. and van PELT, N.: Properties of infectious ribonucleic acid from wheat streak mosaic virus. *Virology* **42**: 699-706. 1970
5) CHANG, C. A., HIEBERT, E. and PURCIFULL, D. E.: Analysis of *in vitro* translation of bean yellow mosaic virus RNA: Inhibition of proteolytic processing by antiserum to the 49K nuclear inclusion protein. *J. gen. Viol.* **69**: 1117-1122. 1988
6) DOMIER, L. L., FRANKLIN, K. M., SHAHAKUDDIN, M., HELLMANN, G. M., OVERMEYER, J. H.,

HIREMATHS. T., SIAW, M. F. E., LOMONOSSOF, G. P., SHAW, J. G. and RHOADS, R. E.: The nucleoted sequence of tobacco vein mottling virus RNA. *Nucleic Acid Res.* **14**: 5417-5430. 1986

7) DOUGHERTY, W. G. and HIEBERT, E.: Translation of potyvirus RNA in a rabbit reticulocyte lysate: cell-free translation strategy and genetic map of the potyviral genome. *Virology* **104**: 183-194. 1980
8) DOUGHERTY, W. G., ALLISON, R. F., PARKS, T. D., JOHNSTON, R. E., FEILD, M. J. and ARMSTRONG, F. B.: Nucleotide sequence at the 3' terminal of pepper mottle virus genmic RNA: Evidence for an alternative mode of potyvirus capsid protein gene organaization. *Virology* **146**: 282-291. 1985
9) DOUGHERTY, W. G. and CARRINGTON, J. C.: Expression and function of potyviral gene products. *Ann. Rev. Phytopathol.* **26**: 123-143. 1988
10) GOLDBACH, R. and van KAMMEN, A.: Structure, replication, and expression of the bipartite genome of cowpea mosaic virus. *in* Plant Molecular Virology (Davies, J. W. edt) CRC Press Inc. **2**: 83-120. 1985
11) GOLDBACH, R.: Genome similarities between plant and animal RNA virus. *Microbiological Sciences.* **4**: 197-202. 1987
12) GOUGH, K. H., AZAD, A. A., HANNA, P. J. and SHUKLA, D. D.: Nucleotide sequence of the capsid and nuclear inclusion protein genes from the Jhonson grass strain of sugarcane mosaic virus RNA. *J. gen. Virol.* **68**: 297-304. 1987
13) GUBLER, U. and HOFFUMAN, B. J.: A simple and very efficient method for generating cDNA libraries. *Gene* **25**: 263-269 1983
14) HAMMOND, J. and HAMMOND, R. W.: Molecular cloning, sequencing and expression in *Eschrichia coli* of the bean yellow mosaic virus coat protein. *J. gen. Virol.* **70**: 1961-1974. 1989
15) HANAHAN, D.: Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* **166**: 557-580. 1983
16) HARI, V., SIEGEL, A., ROZEK, C. and TINBERLAKE, W. E.: The RNA of tobacco etch virus contains poly (A). *Virology* **92**: 568-571. 1979
17) HELLMANN, G. M., SHAW, J. G., KESNAW, J. A., CHU, K. Y., PIRON, T. P. and RHOADS, R. E.: Cell free traanslation of tobacco vein mottling virus RNA. *Virology* **106**: 207-216. 1980
18) HELLMANN, G. M., HIREMATH, S. T., SHAW, J. G. and ROADS, R. E.: Cistron mapping of tobacco vein mottling virus. *Virology* **151**: 159-171. 1986
19) HENIKOFF, S.: Unidirectional digestion with exonuclease III creats targeted breakpoints for DNA sequencing. *Gene* **28**: 351-359. 1984
20) HIEBERT, E., TREMAINE, J. H. and RONALD, W.: The effect of limited proteolysis on the amino acid composition of five potyviruses and on the serological reaction and peptide map of the tobacco etch virus capsid protein. *Phytopathology* **74**: 411-416. 1984
21) HOLLINGS, M. and BRUNT, A. A.: Potyvirus. *in* Handbook of Plant Virus Infection: Comparitive Diagnosis (Kurstak, E. edt). Elservier/Noth Holland Biomedical, Amsterdam. pp 731-809. 1981
22) KITAMURA, N., SEMLER, B. L., ROTHBERG, P. G., LARSEN, G. R., ADLER, C. J., DORNER, A. J., EMINI, E. A., HANECAK, R., LEE, J. J., van der WERF, S., ANDERSON, C. W. and WIMMER, E.: Primary structure, gene organization and polupepetide expression of poliovirus RNA.

Nature (London) **291**: 547-552. 1981

23) KUME, H., TANAKA, S. and MURAYAMA, D.: A strain of bean yellow mosaic virus isolated from mosaic-diseased red clover plant. *Mem. Fac. Agr. Hokkaido Univ.* **7**: 435-449. 1970

24) LAEMMLI, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**: 680-685. 1970

25) LAIN, S., RIECHMANN, J. L., MÉNDEZ, E. and GARCIA, J. A.: Nucleotide sequence of the 3' terminal region of plum pox potyvirus RNA. *Virus Reseach* **10**: 325-342.

26) LIPSCOMB, E. L., HORTON, H. R. and ARMSTRONG, F. B.: Molecular weight, subunit structure, and amino acid composition of the branched chain amino acid amino transferase of *Salmonella tryphimum*. *Biochemistry* **13**: 2070-2077.

27) MAISS, E., TIMPE, U., BRISSKE, A., JELKMANN, W., CASPER, R., HIMMLER, G., MATTANOVICH, D. and KATTINGER, H. W. D.: The complete nucleotide sequence of plum pox virus RNA. *J. gen. Virol.* **70**: 513-524. 1989

28) MANIAITIS, T., FRITSCH, E. F. and SAMBROOK, J.: Procedures for cDNA cloning. *in* Molecular Cloning: A Laboratory Manual, pp. 231-242. New York: Cold Spring Harbor Loboratory.

29) MESSING, J.: New M13 vectors for cloning. *in* Methods in Enzymology, *Vol.* **101**: 20-78. 1983

30) NICKLIN, M. J. H., TOYODA, H., MURRAY, M. G. and WIMMER, E.: Proteolytic processing in the replication of polio and related viruses. *Biotechnology* **4**: 33-42. 1986

31) RAVELONANDRO, M., VARVERI, C., DELBOS, R. and DUNEZ, J.: Nucleotide sequence of the capsid protein gene of plum pox viruses. *J. gen. Virol.* **69**: 1509-1516. 1988

32) SÄNGER, H. L., RAMM, K., DOMDEY, H., GROSS, H. J., HENICO, K. and RIESNER, D.: Conversion of circular viroid molecules to linear strands. *FEBS Letters* **99**: 117-122. 1978

33) SIAW, M. F. E., SHAHABUDDIN, M., BALLARD, S., SHAW, J. G. and RHOADS, R. E.: Identification of a protein covalently linked to the 5'terminus of tobacco vein mottling virus RNA. *Virology* **142**: 134-143. 1985

34) SHUKLA, D. D., INGLIS, A. S., MCKERN, N. M. and GOUGH, K. H.: Coat protein of potyviruses. 2. Amino acid sequence of the coat protein of potato virus Y. *Virology* **152**: 118-125. 1986

35) SHUKLA,, D. D., STRIKE, P. M., TRACY, S. L., GOUGH, K. H. and WARD, C. W.: The N and C termini of the coat protein of potyviruses are surface-location and the N terminus contains the major virus-specific epitopes. *J. gen. Virol.* **69**: 1497-1508. 1988

36) SHUKLA, D. D. and WARD, W.: Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. *J. gen. Virol.* **69**: 2703-2710. 1988

37) SHUKLA, D. D., JILKA, J., TOSIC, M. and FORD, R. E.: A novel approach to the serology of potyviruses involving affinity-purificated polyclonal antibodies directed towards virus-specific N termini of coat protein. *J. gen. virol.* **70**: 13-23. 1989

38) SOUTHERN, E. M.: Detection of specific sequences among DNA frgments separated by gel electrophresis. *J. Mol. Biol.* **98**: 503-517. 1975

39) UYEDA, I., KOJIMA, M. and MURAYAMA, D.: Purification and serology of bean yellow mosaic virus. *Ann. Phytopath. Soc. Japan.* **41**: 192-203. 1975

40) VANCE, V. B. and BEACHY, R. N.: Translation of soybean mosaic virus RNA *in vitro*: Evidence of protein processing *Virology* **132**: 271-281. 1984

41) van der VLUGT, R., ALLIFS, S., HAAN, P. D. and GOLDBACH, R.: Nucleotide sequence of the 3'-ternimal region of potato virus YN RNA. *J. gen. Virol.* **70**: 229-233. 1989
42) van WEZENBEEK, P., VERUER, J., HARMSEN, J., VOS, P. and vas KAMMEN, A.: Primary structure and gene organization of the middle-component RNA of cowpea mosaic virus. *EMBO J.* **2**: 941-946. 1983
43) WILLINK, J. and van KAMMEN, A.: Proteases involved in the processing of viral polyproteins. *Arch. Virol.* **98**: 1-26. 1988