Nucleotide Sequence at the 3′-Terminal Region of Sweet Potato Feathery Mottle Virus (Ordinary Strain, SPFMV-O) RNA†

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Received November 10, 1993

Note

cDNA encoding the 3′-terminal region of sweet potato feathery mottle virus (ordinary strain, SPFMV-O) RNA was cloned and sequenced. The 2324-bp cDNA contained an open reading frame (ORF) of 2066 bp followed by an 3′ non-coding region and a poly(A) region. The ORF covered the coat protein and the carboxy terminus of the nuclear inclusion ‘b’ protein (NIB).

Sweet potato feathery mottle virus ordinary strain, (SPFMV-O), previously described as Mo-isolate,3 is a widely distributed potyvirus throughout the sweet potato growing area in Japan.4 Generally, potyviruses have a single-stranded, positive-sense RNA genome with a poly(A) sequence at its 3′ end, which is translated into one large polyprotein that is cleaved into smaller polypeptides. The coat protein cistron is at the 3′-terminal region of the genome.5 In this report, we describe the cDNA cloning and nucleotide sequencing of the 3′-terminal region of sweet potato feathery mottle virus (ordinary strain, SPFMV-O) RNA.

We have purified virus particles from infected Ipomoea nil leaves (60–100 g), which were stored at −85 °C as previously described5 and prepared genomic RNA by a previously reported method6 with a slight modification, that the RNA was prepared by phenol extraction followed by ethanol precipitation instead of sucrose gradient ultra-centrifugation. Two µg of RNA was used for cDNA synthesis. cDNA was synthesized by the method of Gubler and Hoffman7 using a cDNA Synthesis Kit (Pharmacia-LKB) and oligo(dT)12-18 primer, and cloned into the CIP-treated EcoRI site of pBluescript II SK+(Stratagene). After agarose gel electrophoresis of plasmid DNAs prepared by the rapid preparation method,8 one clone designated pMO-0.8 was found to carry an 0.8-kb insert. This cDNA insert was labelled with 32P-dCTP and used as a probe for Northern hybridization and colony hybridization. It reacted with RNA from SPFMV-O infected leaves of I. nil and not with that from healthy leaves. By colony hybridization, one clone designated pMO-2.3 with a longer cDNA insert of 2.3 kb was isolated and characterized in detail.

The dideoxy nucleotide sequence analysis demonstrated that pMO-2.3 contained 2324 bp of the cDNA (including a 34-bp poly(A) sequence) corresponding to the 3′-terminal region of the SPFMV-O genome. One long ORF encoding 688 amino acids was found (Fig. 1). The amino acid sequence (453 to 462) completely coincided with the reported amino acid sequence of a peptide derived from the trypsin-treated coat protein of SPFMV isolated in Israel.9 By analogy to the protease cleavage sites of other potyviruses,10 the cleavage site between nuclear inclusion ‘b’ protein (NIB) and the coat protein was predicted as shown in Fig. 1. The resulting coat protein is 315 amino acids in length and the 3′-non-coding region is 224 nucleotides excluding the poly(A) sequence.

The 3′ non-coding region does not contain the signal sequence, AAUAAA, for poly(A) tailing. However, the sequence UAUGU, important for termination in yeast,11 is in the region from 2097 to 2101. All the reported sequences of the 3′ non-coding region of potyviruses do not contain this sequence. Thus, it remains obscure whether the sequence is important or not. In addition, we found a unique predicted secondary structure at the 3′-terminal region of the genomic RNA, with 3′ terminal 21 nucleotides together with the poly(A) sequence forming the secondary structure (Fig. 2). The ΔG value for the structure was calculated to be −10.9 kcal using the DNASTAR program (Hitachi Software Engineering Co.) based on the algorithm by Zuker and Stiegler.8 The secondary structure may be involved in replicate recognition or binding. In other potyviruses, secondary structures in the 3′-non-coding region were reported in pepper mottle virus9 and tobacco vein mottling virus10 however, they do not contain poly(A) sequence.

Upstream from the cleavage site, a sequence coding for 373 amino acids corresponds to the carboxy-terminal region of the NIB. In this sequence of NIB, the consensus amino acids responsible for the putative RNA polymerase of RNA viruses12 are found (Fig. 1).

Nucleotide sequences of the coat protein cistron have been reported for two SPFMV strains (RC and C) isolated in the U.S.A.13–15 The coat protein cistron of SPFMV-O was 93% and 80% identical at the nucleotide level, and 96% and 83% identical at the amino acid level, to corresponding cistrons of RC and C strain, respectively. Figure 3 shows the alignment of the amino acid sequences of those three strains. Major sequence differences were found in N-termini. The 3′-non-coding region of SPFMV-O genome was two bases longer than those of RC and C. However, it showed high similarity to RC (96%) and C (98%) as in the case between RC and C (98%). Nucleotide sequences necessary for the 3′-terminal secondary structure are completely conserved both in RC and C.

Acknowledgments. This work was supported by a Grant-in-Aid for Biotechnological Plant Breeding Research Program from the Ministry of Agriculture, Forestry, and Fisheries of Japan.

References

† The nucleotide sequence data reported will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases under the accession number D16664.

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Fig. 1. Nucleotide Sequence of the cDNA Insert in the Clone pMO-2.3 and Deduced Amino Acid Sequences of the Carboxy-terminal Region of Nib Protein and the Coat Protein.

Arrowhead indicates the cleavage site. Underlined region coincides with the 10 amino acid sequence of a peptide derived from tryptic-treated coat protein of SPF-MV isolated in Israel. Asterisks below the amino acids indicate the consensus amino acids for RNA polymerase of RNA viruses. Arrows below nucleotide sequence indicate the inverted repeat (see Fig. 2).
Nucleotide Sequence of Sweet Potato Feathery Mottle Virus

G A
U U
2280 G C
C G
U A
U G
G C
G C 2290
U A
U A
U A
U A
S' 2270 U A 3'
A A C C A A A A (A)n

Fig. 2. Predicted Secondary Structure at the 3'-Terminal Non-Coding Region.

The \( \Delta G \) value calculated by the DNASIS program (Hitachi Software Engineering Co.) is \(-10.9\) kcal.


Fig. 3. Comparison of Coat Proteins of SPFMV-O, RC, and C Strains. Bars indicate identical amino acid residues.