Characterization of cocksfoot mottle sobemovirus genomic RNA and sequence comparison with related viruses

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The genome of cocksfoot mottle virus (CfMV) is a positive-sense ssRNA molecule of 4082 nucleotides as revealed by sequencing the entire genome. The 5'-untranslated region of the genome is 69 nucleotides and the 3'-untranslated region is 225 nucleotides in length. The coding region contains four open reading frames (ORFs). The organization of CfMV ORFs differs significantly from that of the previously sequenced sobemoviruses southern bean mosaic virus and rice yellow mottle virus. ORF1 encodes a protein having a calculated molecular mass of 12.3 kDa. The function of this protein is unknown. The next ORF codes for the putative VPg and serine protease. The ORF2a product consists of 568 amino acids, with a calculated molecular mass of 60.9 kDa. The replicate of CfMV is translated as part of a polyprotein by −1 ribosomal frameshifting in ORF2a. The calculated molecular mass of the trans-frame protein is 103.4 kDa. ORF3 encodes the 27.6 kDa coat protein. This has been verified by amino acid sequencing of the CfMV coat protein N terminus. Northern blots of total RNA from CfMV-infected barley leaves reveal the 4.1 kb genomic RNA band and one virus-specific band of 1.2 kb, which may represent a subgenomic RNA for coat protein synthesis.

Introduction

Cocksfoot mottle virus (CfMV) is a member of the sobemovirus group (Rybicki, 1991). The virus particles are isometric and 30 nm in diameter. The CfMV genome is a monopartite ssRNA molecule with an Mₐ of 1.45x10⁶. Its main host is cocksfoot (Dactylis glomerata), an important herbage grass in the Nordic countries. Wheat, oat and barley are experimental hosts. The entire nucleotide sequence of the RNA genome has been reported for three members of the sobemovirus group: the cowpea strain of southern bean mosaic virus (SBMV-C; Wu et al., 1987), the bean strain of SBMV (SBMV-B; Othman & Hull, 1995) and rice yellow mottle virus (RYMV; Ngon A Yassi et al., 1994). Recently we have reported that the CfMV genome organization differs substantially from that of SBMV-C and RYMV, as its polyprotein is translated from two different open reading frames (ORFs) via −1 ribosomal frameshifting (Mäkinen et al., 1995). In this paper the entire nucleotide sequence of CfMV is reported and compared to related viruses. The possible origins of the in vitro translation products produced from CfMV genomic RNA are discussed.

Methods

Virus purification and nucleic acid extraction. CfMV origin, propagation and the purification of virions were as described earlier (Mäkinen et al., 1995). Viral RNA was purified by treating the virus with proteinase K and SDS (Dougherty & Hiebert, 1980) followed by phenol–chloroform extraction and ethanol precipitation (Puurand et al., 1992).

CfMV cDNA synthesis, cloning, sequencing and analysis. CfMV cDNA synthesis and cloning in the Lambda gt11 Vector (Promega), screening of the resulting library, isolation of CfMV-specific cDNA clones and their sequencing have been described (Mäkinen et al., 1995). Both subcloning using standard methods (Sambrook et al., 1989) and generation of nested unidirectional deletions with exonuclease III (Nested Deletion Kit; Pharmacia) were used to make clones suitable for sequencing. Genetics Computer Group programs (University of Wisconsin; Devereux et al., 1984), DNA Strider (Commissariat a l’Energie Atomique, France), DNAid* (Ecole Polytechnique, France), Clustal V (Higgins et al., 1992) and PCGENE (University of Geneva, Switzerland) were used for sequence analysis.

N-terminal amino acid sequence of the coat protein. The coat protein of CfMV was separated out by SDS-PAGE (12.5% gel) as described by Laemmli (1970). The gel was aged for 3 days at 4 °C prior to electrophoresis (Staunton et al., 1989). The coat protein was electro-

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Fig. 1. For legend see opposite.
Fig. 1. The complete nucleotide sequence of the CfMV genomic cDNA. The amino acid sequences of the ORFs 1, 2a, 2b and 3 are presented under the nucleotide sequence in single letter code. The slippery heptanucleotide used for the -1 ribosomal frameshift is shown in bold.
transferred onto PVDF membrane and visualized by staining with Coomassie brilliant blue (Staunton et al., 1989). The band corresponding to the coat protein was excised and applied to the reaction cartridge of a gas-pulsed-liquid sequencer equipped with an on-line phenylthiohydantoin amino acid analyser (Kalkkinen & Tilgmann, 1988).

PCR amplification of the cDNA. Some clones were obtained by PCR amplification. Both single- and double-stranded cDNA were used as templates for the PCR amplification using a Perkin Elmer DNA Thermal Cycler or MJ Research Minicycler. The reaction mixture contained 10 mM-Tris·HCl pH 8.8, 1.5 mM-MgCl₂, 50 mM-KCl and 0.1% Triton X-100, 0.1 mM-dNTP, 80 pmol of each primer, 50 ng of template cDNA and 2 units of DynaZyme DNA polymerase (Finnzymes) or Taq DNA polymerase (Perkin Elmer, Fermentas). After denaturation of the DNA at 95 °C for 5 min, the reaction mixtures were subjected to 30 cycles of 1.5 min at an annealing temperature calculated for each primer pair used, 2.5 min at 72 °C and 1.5 min at 95 °C. The PCR fragments obtained were cloned into vectors pGEM-T (Promega), pCR1000 (Invitrogen) or pUC57 (Fermentas).

Cloning of the 5’ and 3’ ends. The 5’-terminal sequence of CfMV was determined using the PCR product. The first strand of cDNA was synthesized using an oligodeoxyribonucleotide complementary to nucleotides 357–380 in the CfMV sequence: 5’ CGGCTGTAACATAATCCACGTT (primer #4615). Single-stranded DNA was purified with a Sephalogel PhagePrep Kit (Pharmacia) or Wizard DNA Clean-Up System (Promega) and a poly(A) tail was added by terminal transferase (Promega). The DNA product was amplified by PCR using (dT)₁₃dN as the second primer.

To clone the 3’ end, 1 µg of viral RNA was polyadenylated with 5 units of poly(A) polymerase (Pharmacia) according to the manufacturer’s instructions. First-strand synthesis of cDNA was primed with 5’ AACTGGAGAATTCCGCGGCGCAAGA(T)₁₈ oligodeoxyribonucleotide. The cDNA was then subjected to PCR using the same primer and a specific primer 5’ CAGGATCTTGCTCAAGGGATTCATGTCACG (primer #4614: nucleotides 3812–3835 in the CfMV sequence).

Primer extension. Three µg of CfMV RNA was subjected to primer extension using the oligodeoxyribonucleotide primer 5’ CTAGCTAC- TCTTACACCTC, located 50–70 nucleotides from the 5’ terminus of the obtained 5’-end clones. These clones were also used to provide the size markers for the urea–polyacrylamide gel by using the same primer for chain-termination sequencing reactions (Sambrook et al., 1989).

Isolation of the total RNA and Northern blot analysis of the subgenomic RNA. Total RNA from CfMV-infected barley (cv. Lise) leaves and from control barley leaves was isolated using the method described by Logemann et al. (1987). Electrophoresis in formaldehyde-containing agarose gels and hybridizations were done according to the blotting and hybridization protocols for Hybond membranes (Amersham). Blotting of the gel onto Hybond-N nylon filters was done after alkali transfer of total RNA as described by Low & Rausch (1994). Eight µg of total RNA was loaded per lane. A probe covering 364 3’-terminal nucleotides of CfMV ORF3 was randomly primed (Feinberg & Vogelstein, 1983) and labelled with [³²P]dCTP (NEN).

Results and Discussion

The CfMV cDNA clones picked up from the library or obtained by PCR were sequenced on both strands. The sequence of 98.2% of the genome was obtained from at least two independent clones. The complete nucleotide sequence of CfMV RNA was 4082 nucleotides long, being the shortest sequenced sobemovirus (Fig. 1). Terminal sequences were verified by polyadenylation of the termini prior to PCR amplification. The nucleotides determined by primer extension matched the sequences obtained with 5’-end clones (Fig. 2). The 3’-end sequence was proven by sequencing three cDNA clones obtained by RT–PCR of three independently isolated and in vitro polyadenylated CfMV RNAs. All of the sequences were identical. The 5’-untranslated region was 69 nucleotides and the 3’-untranslated region was 225 nucleotides in length.

Coding capacity of CfMV

The CfMV genome contains four ORFs (Fig. 3), which are compact and overlap each other, except that between ORFs 1 and 2a there is a 32 nucleotide intergenic region. The A of the first AUG is located at nucleotide 70. The context of this AUG is poor when compared to the consensus sequence for translation initiation in plants, AACAAUGGC, described by Lütcke et al. (1987). However, as in RYMV, this is the only translation initiation codon in any frame upstream from ORF2a. ORF1 encodes a protein with 109 amino acids and a calculated molecular mass of 12.3 kDa. In vitro translation of CfMV genomic RNA revealed four protein products of 100, 71, 34 and 16 kDa (Mäkinen et al., 1995). The smallest protein may represent the ORF1 product. Its function is unknown, but Othman & Hull (1995) have suggested a movement protein function. CfMV ORF1, at nucleotides 236–312, has 59.2% identity with the beet yellows closterovirus 65 kDa protein gene, which is related to the heat shock protein 70 gene. The 65 kDa protein is believed to provide a virus transport function (Agranovsky et al., 1994).

CfMV, in contrast to other fully sequenced sobemoviruses, lacks the continuous large ORF that codes for the putative VPg, protease and replicase. Instead it has two overlapping ORFs, called 2a and 2b, which code for these proteins (Fig. 3). In the CfMV genome, ORF2a begins 32 nucleotides after the stop codon for ORF1. ORF2a comprises 1710 nucleotides (from 424 to 2133) and encodes a protein of 568 amino acids with a calculated molecular mass of 60.8 kDa. ORF2b is in the -1 reading frame compared to ORF2a. Consensus signals for a -1 ribosomal frameshifting event (Jacks et al., 1988) can be found at the beginning of the nucleotides that overlap in ORF2a and ORF2b: the slippery heptanucleotide sequence 5’ UUUAAAC and a stem–loop structure just downstream. We showed previously that the putative replicase of CfMV is translated in vitro as a part of a polyprotein by -1 ribosomal frameshifting (Mäkinen et al., 1995). ORF2b has 1650 nucleotides (1603–3253) and encodes a 56.3 kDa protein comprising 504 amino acids from the first AUG. The
transframe protein is 942 amino acids long and has a calculated molecular mass of 103.4 kDa. The size of the transframe protein and the largest CIMV RNA \textit{in vitro} translation product correlate well (Mäkinen \textit{et al.}, 1995).

The N-terminal part of the ORF2a-encoded protein is presumably the VPg by analogy to the location of the putative VPgs of other sequenced sobemoviruses. In addition to the conserved 14 and 9 amino acid blocks proposed by Ngon A Yassi \textit{et al.} (1994), the sequence motif GxPxFDPxYG can be found in the putative VPg of each of these viruses (Figs 3 and 4). Despite the fact that these three motifs are well conserved among all the sobemoviruses, their location in the putative VPg domain is different (Fig. 4). In general, no common sequence elements in plant viral VPgs have been found (Gorbalenya & Koonin, 1993). However, an amino acid triplet FDP is present in the VPg of the potyviruses tobacco etch virus (Allison \textit{et al.}, 1986; Murphy \textit{et al.}, 1991), potato Y virus (Robaglia \textit{et al.}, 1989) and potato A virus (Puurand \textit{et al.}, 1994). The significance of motif GxPxFDPxYG is unknown.

The next protein coded by ORF2a has features of a typical sobemovirus/luteovirus serine protease. It has the H and D residues common to all serine proteases, and an S at the site where the picornavirus 3C protease has C in its catalytic centre (Gorbalenya \textit{et al.}, 1988). E/S and E/T cleavage sites were proposed for the sobemovirus serine proteases by Gorbalenya \textit{et al.} (1988).

There are several putative cleavage sites in the ORF2a-coded protein. One putative protease cleavage site, located after the protease consensus sequence, is conserved among the sobemoviruses SBMV-C, RYMV...
Calculated molecular masses of 35.6 and 68.6 kDa. In poor context for the initiation of protein synthesis, it is highly conserved WAD/WGD amino acid sequence of a 34 kDa protein, which correlates well with the joint product from this ORF. The theoretical size of the protein of ORF2a, contains the GDD motif typical of the replicase produced using the E/T cleavage site in front of the putative or proven -1 frameshift signal. When the translation mixture was subjected to in vitro translation of the viral RNA. The coat protein of SBMV is translated from a subgenomic RNA molecule, which is also present within the virus particles (Ghosh et al., 1981). In contrast, it was not possible to detect any subgenomic RNA enclosed in CfMV particles in Northern blot hybridization (Fig. 6). When the in vitro translation mixture was subjected to Western blot analysis no coat protein signal was seen, while coat protein isolated from purified virus particles was easily detected (Tamm, 1994). For these reasons it is more likely that the 34 kDa translation product is a cleavage product of the polyprotein.

In Northern blots of total RNA of CfMV-infected leaves, two virus-specific bands of 4.1 kb and 1.2 kb were detected (Fig. 6) using a probe from the C-terminal part of ORF3 coding for the coat protein. A total RNA sample from uninfected barley did not give a CfMV-specific signal. Whether the 1.2 kb CfMV-specific RNA molecule is the subgenomic RNA for coat protein synthesis and the true initiation site for the synthesis of CfMV subgenomic RNA remains to be determined. As the translation initiation site of CfMV coat protein is 989 nucleotides upstream from the 3' end of the genome, a subgenomic RNA of 1-2 kb seems highly possible.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Nucleotides</th>
<th>Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>CfMV</td>
<td>26</td>
<td>VSVAMAAMTDSPPTLICM-GILVSVLNVWCAVWOEASELILGVSL</td>
</tr>
<tr>
<td>RYMV</td>
<td>27</td>
<td>AGLIAAIVSERLPLYTVASIWPAAHASNVSRSRFEVGEVIEI</td>
</tr>
<tr>
<td>SBMV-C</td>
<td>24</td>
<td>APIYGSDPSPACEIPAVMTLATGMLGSTSVSFGTRYVRVYRSPESK</td>
</tr>
<tr>
<td>SBMV-B</td>
<td>25</td>
<td>FDIHRGVNTPAHYIPAVILPSLCL-VERVIRDIPYKVVRTRL---L</td>
</tr>
</tbody>
</table>

**Fig. 4.** Amino acid alignment of the putative VPg regions of CfMV, RYMV, SBMV-C and SBMV-B. The conserved sequence motif GxPxFDPxYG is indicated. The 14 and 9 amino acid motifs proposed by Ngon A Yassa et al. (1994) are underlined. CONS. conserved amino acids; (*), conserved in all sequences shown; (!), partially conserved.

and CfMV (Wu et al., 1987; Ngon A Yassi et al., 1994; Fig. 3) and other viruses; potato leafroll virus (PLRV) and beet western yellows virus (BWYV) (both belonging to luteovirus subgroup II; Mayo et al., 1989; Veidt et al., 1988), pea enation mosaic virus (PEMV; Demler & de Zoeten, 1991), mushroom bacilliform virus (MBV; Revill et al., 1994) and a human astrovirus (H-Ast2; Jiang et al., 1993). The use of this E/S cleavage site in CfMV polyprotein processing would produce proteins with calculated molecular masses of 35.6 and 68.6 kDa. In vitro translation of the CfMV genomic RNA revealed a protein of 34 kDa, which correlates well with the joint size of VPg and the protease.

In the ORF2a protein product we have located a highly conserved WAD/WGD amino acid sequence followed by a D/E-rich domain. This amino acid motif or a very similar one can also be found in SBMV, RYMV, PLRV, BWYV, PEMV, MBV and H-Ast2 in front of the putative or proven -1 frameshift signal (Figs 3 and 5). Its function is unknown. The remainder of the ORF2a-encoded protein is very basic.

The amino acid sequence of the putative replicase, encoded by ORF2b, contains the GDD motif typical of RNA-dependent RNA polymerases (Kamer & Argos, 1984) near the C terminus of the protein (Fig. 3). In vitro translation of the CfMV RNA results in a 70 kDa product from this ORF. The theoretical size of the replicase produced using the E/T cleavage site in front of the replicase is 60-7 kDa (Fig. 3). If the E/S cleavage site after the protease consensus sequence is exploited instead then the protein product would be 68-6 kDa. This could be the 70 kDa product seen on SDS-PAGE.

The mechanism for the translation initiation of ORF2a is currently unknown. Since the AUG for ORF1 is in a poor context for the initiation of protein synthesis, it could permit the 40S ribosomal subunit to bypass ORF1 by leaky scanning (Kozak, 1989) and initiate translation at the first AUG of ORF2a. This AUG is surrounded by a sequence that satisfies the proposed initiation context in plants (AAGAAUGGG versus AACAAUGGC; proposed by Lütcke et al., 1987).
CfMV 343 GGGKAWGDSDDEDTQETAIRPLNYQ
RYMV 384 SGOPSWADRFSGDSEDVDIETSP
SBMV-C 370 PKGKAWAMLDDDLPLLPPKVMNAA
SBMV-B 371 ESGKYWADDDSLPPPKVVDGKM
PLRV 435 LTDKNMADDYDSDEYGLERAATN
BWWV 434 TKGKYWGDDEDDDFSKEKDLGS
PEMV 568 GPILGWADTEDDESAPRSGNGLF
MBV 481 LGFWDWDAPDFDELVFESTMV
H-Ast2 836 SYDFLOWDDEDAKFIAPLPAHRTLKAD

**CONS**

![Fig. 5](image)

**Fig. 5.** Amino acid alignment of the WAD/WGD plus D/E-rich region upstream from putative or proven frameshift signals in CfMV and other viruses

![Fig. 6](image)

**Fig. 6.** Northern blot analysis of total RNA from CfMV-infected barley plants. Lanes 1-3, 8 µg of total RNA isolated from three different CfMV-infected barley plants; lanes 4-6, 8 µg of total RNA from three different healthy barley plants; lane 7, 100 ng of CfMV RNA isolated from purified virus particles; lane 8, 50 ng of CfMV RNA from virus particles. Positions of RNA markers are shown on the left.

**CfMV**

G--AUAAUAGUGCAAGAAGACACA-----------CUUGUAUCGUUCC 37

**RYMV**

A--CAAUUGAAGCUAGGAAAGGAACAUUU---GCAGAAAGCAUCC 41

**SBMV-C**

CACAAAUAUAAGAAGAAAGGAUCGAUUCUACCUCUUGUUC- 46

**SBMV-B**

CACAAAUAUAAGAAGAAAGGAUCGAUUCUACCUCUUGUUC- 46


**Fig. 7.** Multiple alignment of the 5'-end sequences of CfMV, SBMV-C, SBMV-B and RYMV. No clear consensus sequence was detected.

**Sequence similarities to related viruses**

CfMV proteins are remarkably similar to their counterparts in SBMV and RYMV, although there are substantial differences in genome organization. The intergenic region between ORF1 and ORF2a is present in CfMV, RYMV and SBMV-B genomes but is absent in SBMV-C. SBMV-C and RYMV have ORF3 nested in the ORF2 sequence but in another reading frame. The CfMV genome lacks the analogous ORF. ORF3 in the CfMV genome codes for the coat protein.

The lengths of CfMV untranslated termini are similar to those of RYMV: CfMV 5', 69 nucleotides; 3', 225 nucleotides; and RYMV 5', 79 nucleotides; 3', 245 nucleotides. In the SBMV-C genome the untranslated regions have been reported to be much shorter (48 and 138 nucleotides, respectively). The 5'-untranslated region of CfMV shares 62%, 42.5% and 58.3% identical nucleotides with the corresponding regions of SBMV-C, SBMV-B and RYMV, respectively. However, multiple alignment of the 5'-end sequences of CfMV, SBMV-C, SBMV-B and RYMV reveals only 14.1% identity (Fig. 7). We were not able to detect any clear consensus sequence from sobemovirus 5'-untranslated regions. Obvious sequence similarities were also not found in the 3'-untranslated regions of CfMV, SBMV-C, SBMV-B and RYMV. Secondary structure analysis of the CfMV 3'-untranslated region with RNAFOLD (Devereux et al., 1984) did not reveal the presence of a tRNA-like structure, which can be found in the 3'-untranslated region of RYMV (Ngon A Yassi et al., 1994).

ORF1 of CfMV is unrelated to the corresponding
ORFs of other sequenced sobemoviruses. The putative ORF2a-encoded proteins (VPg, protease and the amino acid sequence after the protease) were separately compared to the polyproteins of SBMV-C, SBMV-B and RYMV. The CfMV ORF2a product was divided into separate parts using the putative E/T and E/S cleavage sites before and after the protease consensus sequence. The potential VPg region was 30% identical between RYMV and CfMV, 23% identical between CfMV and SBMV-C and 19% identical between CfMV and SBMV-B while the corresponding values for the protease region were 37%, 38% and 28%, respectively. Several cellular proteases and proteases of picorna-, poty- and comoviruses have H, D and C or S residues in their catalytic sites (Gorbalenya et al., 1988). These important amino acids can be found in the CfMV serine protease, located exactly as in the RYMV amino acid sequence: H is separated from D by 32 amino acids and the distance between D and S is 66 amino acids. Comparison of the ORF2a amino acid sequence after the WAD/WGD plus D/E-rich region is complicated because in this region CfMV does not have the replicate as in SBMV-C and RYMV, but instead has the end of ORF2a which overlaps with ORF2b. This kind of genome organization resembles that of luteovirus subgroup II. The 5' end of CfMV ORF2b contains signals for ribosomal -1 frameshifting (Mäkinen et al., 1995). Similar frameshifting consensus signals can be found in the beginning of ORF3 of SBMV-C and RYMV. ORF3 is nested in the ORF2 sequence of these two viruses but in another reading frame and its function is unknown. Amino acid comparisons between the putative replicates of CfMV and the other sequenced members of sobemo- and luteovirus subgroup II viruses have been reported by us earlier (Mäkinen et al., 1995).

Although SBMV-B had an overall similarity to SBMV-C and RYMV, it lacked the analogue of ORF3 of these two viruses. Its genome organization resembles that of CfMV. However, SBMV-B polyprotein was reported to be translated from a single ORF without ribosomal frameshifting. Interestingly, the SBMV-B genome also contains a putative -1 ribosomal frameshift signal: slippery sequence UUUAAAC starting at position 1728 followed by a strong stem-loop structure after 7 additional nucleotides.

Comparison of sobemovirus coat proteins revealed 23% identity between CfMV and SBMV-C, 23% identity between CfMV and SBMV-B and 35% identity between CfMV and RYMV. SBMV-C and RYMV have 29% identical amino acids. CfMV has, in common with the other sequenced sobemoviruses, a highly basic amino acid sequence at the N terminus of the coat protein. This region in CfMV resembles the bipartite signal proposed for nuclear targeting (Dingwall & Laskey, 1991). The significance of this finding is presently not known, but CfMV particles have been reported to be present in the cell nucleus (Chamberlain & Catherall, 1976).

Conclusions

CfMV is a member of the sobemovirus group based on the sequence similarities between the polyproteins and coat proteins of CfMV, SBMV and RYMV. CfMV is more closely related to RYMV than SBMV-C and SBMV-B. However, the genome organization of CfMV is significantly different from other characterized sobemoviruses. It is more closely related to SBMV-B, as both lack the ORF3 of SBMV-C and RYMV. The polyprotein-coding region of CfMV is more similar to that of subgroup II luteoviruses, PLRV and BWYV, than to the reported genomic organizations of sobemoviruses. The evolution of subgroup II luteoviruses has been proposed to have occurred by a recombination event between a sobemovirus and a subgroup I luteovirus (Miller et al., 1995). The polyprotein in this recombination evolved from the sobemovirus counterpart. The finding that the replicase of the CfMV is translated, in a similar way to the subgroup II luteoviruses, as part of the polyprotein by -1 ribosomal frameshifting supports this model.

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References
Gene sequence of potato leafroll luteovirus RNA. Journal of General Virology 70, 1037–1051.


