Localization of functional regions of the cucumber mosaic virus RNA replicase using monoclonal and polyclonal antibodies

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Monoclonal antibodies were produced using a purified cucumber mosaic virus (CMV) replicase complex, and Escherichia coli-expressed CMV 1a and 2a proteins, as immunogens. Five out of eight monoclonal antibodies, which bound to the 1a and 2a proteins in immunoblots, inhibited the RNA-dependent RNA polymerase (RdRp) activity of the purified replicase complex in vitro. Epitope mapping showed that two of the inhibitory antibodies interacted with regions of the 1a protein containing putative helicase and methyltransferase domains respectively. Two other inhibitory antibodies mapped to a region of the 2a protein containing the GDD motif which is highly conserved in RdRps. Prior interaction of the latter antibodies with a peptide containing the GDD motif prevented the antibody-mediated inhibition of the replicase. Polyclonal antibodies which inhibited the RdRp activity of the replicase complex were also produced using peptides corresponding to conserved helicase and polymerase motifs in the 1a and 2a proteins. The greatest inhibition was shown by antibodies to a peptide containing the GDD motif. These results demonstrate the functional importance of the identified sequence motifs in CMV RNA replication and indicate that the motifs are located in the replicase complex at positions accessible to antibodies, consistent with roles in interacting with the RNA template, RNA primer and enzyme substrates.

Introduction

Positive-strand RNA viruses of the ‘alpha-like’ supergroup (Goldbach et al., 1991) encode several domains with highly conserved sequence motifs in proteins mediating replication of the virus genome. These include RNA-dependent RNA polymerase (RdRp) (Kamer & Argos, 1984; Habili & Symons, 1989; Poch et al., 1989; Koonin, 1991; Bruenn, 1991), nucleoside triphosphate-dependent helicase (Hodgman, 1988; Gorbalenya & Koonin, 1989; Habili & Symons, 1989) and methyltransferase (Mi et al., 1989; Mi & Stollar, 1990, 1991; Rozanov et al., 1992) domains. Mutational analyses have indicated the importance of all three domains, and of some conserved sequence motifs within the domains, in virus RNA replication (see, for example: Inokuchi & Hirashima, 1987; Kroner et al., 1989, 1990; Traynor et al., 1991; Longstaff et al., 1993; Davenport & Baulcombe, 1993; Mi & Stollar, 1990). However, direct inhibition of replication by binding of antibodies to individual sequence motifs within the domains has not been demonstrated and the locations of the sequence motifs within the three-dimensional structure of the replicase complexes is not known.

In cucumber mosaic virus (CMV), the methyltransferase-like and helicase-like domains lie in the N-terminal and C-terminal regions of the RNA 1-encoded 1a protein respectively (Rezaian et al., 1985; Habili & Symons, 1989; Rozanov et al., 1992), whereas the RNA polymerase-like domain lies within the RNA 2-encoded 2a protein (Rezaian et al., 1984; Koonin, 1991). A purified enzyme complex, isolated from CMV-infected plants and capable of completely replicating CMV RNA, contained the 1a and 2a proteins, as well as one major and several minor host proteins (Hayes & Buck, 1990a, 1993). Here we report that monoclonal and polyclonal antibodies that bind directly, or close to, conserved sequence motifs in each of the three domains inhibit the RNA-dependent RNA polymerase (RdRp) activity of a purified CMV replicase preparation. This confirms the functional importance of these sequence motifs in CMV RNA replication and demonstrates their locations within the replicase complex at positions accessible to antibodies.

Methods

Production and assay of monoclonal antibodies. BALB/c mice were injected subcutaneously with 10 μg of purified replicase complex (Fraction 6; Hayes & Buck, 1990a), or purified 1a or 2a proteins.
expressed in *Escherichia coli* (Hayes & Buck, 1990a), emulsified with an equal volume of Freund's complete adjuvant. After two further injections of the immunogen emulsified with an equal volume of Freund's incomplete adjuvant at 2 week intervals, spleen cells were isolated 1 week later and fused with myeloma cells to produce hybridomas. Hybridoma supernatants were screened by enzyme-linked immunosorbent assay (ELISA) using *E. coli*-expressed 1a and 2a proteins as antigens. Bulk production of monoclonal antibodies was obtained by injecting hybridomas into the peritoneal cavity of BALB/c mice and isolating the antibodies from the ascitic fluid (Harlow & Lane, 1988). Antibodies were tested for their ability to react with the 1a and 2a proteins or portions of these in fusion proteins by SDS-PAGE, immunoblotting (Hayes & Buck, 1990a) and antibody-linked polymerase assay (Van der Meer *et al.*, 1983; Hayes & Buck, 1993).

Production and assay of polyclonal antibodies. Peptides corresponding to conserved domains in the 1a and 2a proteins were coupled to keyhole limpet haemocyanin with *N*-maleimidobenzoyl-*N*-hydroxysuccinimide via the C-terminal cysteine (Liu *et al.*, 1979), which was added if not present in the sequence. Rabbits were injected with 500 μg of the coupled peptide emulsified with an equal volume of Freund's complete adjuvant, followed by further injections of the immunogen emulsified with an equal volume of Freund's incomplete adjuvant at 4 week intervals until a satisfactory response, assayed by the ability of test antisera to interact with the 1a or 2a proteins in immunoblots (Hayes & Buck, 1990a), had been obtained. Antisera were collected 10 days after the last injection and antibodies were isolated as described by Harlow & Lane (1988). Polyclonal antibodies to the *E. coli*-expressed 1a and 2a proteins have been described previously (Hayes & Buck, 1990a).

Antibody-mediated inhibition of RNA-dependent RNA polymerase activity. CMV replicase complex (Fraction 6) was prepared as described by Hayes & Buck (1990a). The activity of this preparation was completely dependent on the addition of CMV RNA; incorporation of [32P]UMP into RNA was similar to that described previously (Hayes & Buck, 1990a). Antibodies were all adjusted to a titre of 1:5000. The purified replicase (10 μl) and antibody (1 to 5 μl) were mixed and incubated at 0 °C for 30 min. CMV RNA (5 μg), 2× RdRp assay buffer (12.5 μl; Hayes & Buck, 1993), bentonite suspension (0.5 μl; Hayes & Buck, 1993) and [32P]UTP (5 to 10 μCi) were then added and the mixture was incubated at 30 °C for 30 min. Incorporation of [32P]UMP into RNA was then assayed by the disc method as described in protocol 7 of Hayes & Buck (1993). The products were also extracted and analysed by gel electrophoresis as in protocols 7 and 10 of Hayes & Buck (1993).

DNA manipulations. Amplification of DNA by the polymerase chain reaction (PCR), DNA cloning and nucleotide sequencing for verification of constructs were as described by Sambrook *et al.* (1989).

Production of fusion proteins. Fusions of the whole or parts of the CMV 1a and 2a genes with the *E. coli* *malE* (malto-β-dextrin protein) gene were made using the vector pMAL-cRI (Fig. 1a) (New England Biolabs), a derivative of pMAL-c (Maina *et al.*, 1988). To clone the whole of the 1a gene into pMAL-cRI, cDNA corresponding to nucleotides 98 to 250 of RNA 1 was amplified by PCR using pCMV1A (Fig. 1b), a full-length cDNA clone of CMV RNA 1 (Hayes & Buck, 1990b), and two oligonucleotide primers, one of which contained an added EcoRI site and the other a BamHI site at the 5' end. After purification with a PrimerErase column (Stratagene), the PCR product was cleaved with EcoRI and BamHI and cloned into the corresponding sites of pMAL-cRI to give pMAL-1a-A. pCMV1A was then cleaved with XbaI and BamHI and the 3.2 kb fragment was cloned into the corresponding sites of pMAL-1a-A to give pMAL-1a-1. To clone the part of the 1a protein corresponding to amino acids 1 to 96 into pMAL-cRI, pMAL-1a-1 was cleaved with XbaI and BamHI and the ends of the 6.6 kb fragment were filled in and ligated together to give pMAL-1a-2. To clone the region of the 1a gene corresponding to amino acids 457 to 992 into pMAL-cRI, the 1.9 kb XbaI-BamHI (blunt-ended) fragment of pCMV1A was cloned into XbaI-HindIII (blunt-ended)-cleaved pMAL-cRI. The resultant clone was cleaved with XbaI and the ends of the linearized plasmid were filled in and ligated together. A clone with the *malE* and 1a sequences in-frame (pMAL-1a-3) was selected by sequencing. To clone the region of the 1a gene corresponding to amino acids 96 to 457 into pMAL-cRI, pCMV1A was cleaved with XbaI and the 1.1 kb fragment was cloned into the XbaI site of pMAL-cRI and a clone with the insert in the desired orientation was selected. To bring the *malE* and 1a genes into the same reading frame, the clone was cleaved partially with XbaI and the overhangs of the ends of the linearized plasmid were digested with micrococcal nuclease. After religation, a clone with the two genes in-frame (pMAL-1a-4) was selected by sequencing. Smaller regions of the 1a gene were amplified by PCR using two primers, one of which had an added EcoRI site and the other a BamHI site at the 5' end and cloned into the corresponding sites of pMAL-cRI.

To clone the whole of the 2a gene into pMAL-cRI, cDNA corresponding to nucleotides 93 to 500 of RNA 2 was amplified by PCR.
using pCMV2A (Fig. 1c), a full-length clone of CMV RNA 2 (Hayes & Buck, 1990b), and two primers one of which contained an added EcoRI site and the other a BamHI site at the 5' end. The purified PCR product was cleaved with EcoRI and BamHI and cloned into the corresponding sites of pMAL-cRI to give pMAL2a-A. pCMV2A was cleaved with NcoI and BamHI and the 2.6 kb fragment was cloned into the corresponding sites of pMAL2a-A to give pMAL2a-1. To clone the part of the 2a gene corresponding to amino acids 1 to 410 into pMAL-cRI, pMAL2a-1 was cleaved with BstII and BamHI and, after end-filling, the ends of the 7.5 kb fragment were ligated together to give pMAL2a-2. To clone the part of the 2a gene corresponding to amino acids 1 to 643 into pMAL-cRI, pMAL2a-1 was cleaved with AsuII and BamHI and, after end-filling, the ends of the 8.2 kb fragment were ligated together to give pMAL2a-3. To clone the part of the 2a gene corresponding to amino acids 644 to 839 into pMAL-cRI, pMAL2a-1 was cleaved with EcoRI and AsuII and, after end-filling, the ends of the 7.2 kb fragment were ligated together. A clone with the malE and 2a genes in the same frame (pMAL2a-4) was identified by sequencing. To clone a part of the 2a gene corresponding to amino acids 410 to 643 into the vector pMAL-cRI and a fusion of the maltose-binding protein and the 2a protein was produced in E. coli DH5α cells containing the recombinant pMAL-cRI/CMV recombinant plasmids were grown to an OD600 of 0.5 and expression of the fusion proteins was induced with IPTG (0-3 mM). After disruption of the cells by sonication, the fusion protein was purified by binding to an amylose resin, followed by elution with maltose (Maina et al., 1988). In the presence of the antibodies, the intensities of both double-stranded and single-stranded RNA bands were reduced in proportion to the degree of inhibition. Hence the inhibition of RdRp activity (Table 1) was due to inhibition of the CMV replicase and not to inhibition of any traces of host RdRp which may have remained in the purified CMV RdRp preparation.

### Epitope mapping of inhibitory monoclonal antibodies that bind to the 2a protein

The gene for the full-length 2a protein was cloned into the vector pMAL-cRI and a fusion of the maltose-binding protein and the 2a protein was produced in E. coli DH5α.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Immunogen</th>
<th>Inhibition* (%)</th>
</tr>
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<tbody>
<tr>
<td>mAB/1a1</td>
<td>Replicase</td>
<td>22-28</td>
</tr>
<tr>
<td>mAB/1a2</td>
<td>Replicase</td>
<td>4-6</td>
</tr>
<tr>
<td>mAB/2a1</td>
<td>Replicase</td>
<td>13-17</td>
</tr>
<tr>
<td>mAB/2a2</td>
<td>Replicase</td>
<td>8-11</td>
</tr>
<tr>
<td>mAB/2a3</td>
<td>Replicase</td>
<td>68-80</td>
</tr>
<tr>
<td>mAB/A</td>
<td>E. coli-expressed 1a protein</td>
<td>7-10</td>
</tr>
<tr>
<td>mAB/B</td>
<td>E. coli-expressed 1a protein</td>
<td>44-50</td>
</tr>
<tr>
<td>mAB/C</td>
<td>E. coli-expressed 2a protein</td>
<td>38-43</td>
</tr>
</tbody>
</table>

* Range of values in three or more replicates.

![Fig. 2. Immunoblotting of 2a fusion proteins. Fusion proteins containing the whole or part of the 2a protein were subjected to electrophoresis in an SDS-polyacrylamide gel, blotted onto Hybond membrane and probed with (a) a polyclonal antiserum to the 2a protein or (b) mAB/2a3. Fusion proteins were produced from: lane 1, pMAL2a-1; lane 2, pMAL2a-2; lane 3, pMAL2a-3; lane 4, pMAL2a-4; lane 5, pMAL2a-5; lane 6, pMAL2a-6; lane 7, pMAL2a-7; lane 8, pMAL2a-8.](image-url)
Regions of the 2a protein used in epitope mapping

Amino acids | Expression vector | Interaction with mAB/2a3
-------------|------------------|----------------------
1–839        | pMAL2a-1         | Yes                  
1–410        | pMAL2a-2         | No                   
1–643        | pMAL2a-3         | Yes                  
644–839      | pMAL2a-4         | No                   
410–643      | pMAL2a-5         | Yes                  
410–530      | pMAL2a-6         | No                   
510–643      | pMAL2a-7         | Yes                  
599–620      | pMAL2a-8         | Yes

Fig. 3. Epitope mapping of monoclonal antibody mAB/2a3. Various regions of the CMV 2a protein (left) were expressed as fusions with the *E. coli* maltose-binding protein using the pMAL vectors indicated. The ability of mAB/2a3 to interact with each fusion protein was assessed by immunoblotting (Fig. 2). The RNA polymerase-like domain (Koonin, 1991) is shown within the 2a protein as a shaded area and the GDD motif is shown as a black box within the polymerase-like domain.

Fig. 4. Ability of fusion proteins containing various regions of the CMV 2a protein to interfere with the inhibition of the CMV RdRp activity by monoclonal antibody mAB/2a3. Fusion proteins 2a-(1–9) were produced in *E. coli* using vectors pMAL2a-(1–9) respectively. Each fusion protein was incubated with mAB/2a3 and each fusion protein/antibody mixture was then incubated with a purified CMV replicase preparation. The effect on RdRp activity was then assayed. The results are expressed as percentages of the RdRp activity without added antibody. A control with mAB/2a3, but not fusion protein added, was included.

Fig. 5. Ability of peptides to interfere with the inhibition of the CMV RdRp activity by monoclonal antibody mAB/2a3. Each peptide was incubated with mAB/2a3 and each peptide/antibody mixture was then incubated with a purified CMV replicase preparation. The effect of RdRp activity was then assayed. The results are expressed as percentages of the RdRp activity without added antibody. A control with mAB/2a3, but not peptide added, was included.
Regions of the la protein used in epitope mapping

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Expression vector</th>
<th>Interaction with mAB/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–992</td>
<td>pMAL1a-1</td>
<td>Yes</td>
</tr>
<tr>
<td>1–96</td>
<td>pMAL1a-2</td>
<td>No</td>
</tr>
<tr>
<td>457–992</td>
<td>pMAL1a-3</td>
<td>No</td>
</tr>
<tr>
<td>96–457</td>
<td>pMAL1a-4</td>
<td>Yes</td>
</tr>
<tr>
<td>96–168</td>
<td>pMAL1a-5</td>
<td>Yes</td>
</tr>
<tr>
<td>168–268</td>
<td>pMAL1a-6</td>
<td>No</td>
</tr>
<tr>
<td>268–457</td>
<td>pMAL1a-7</td>
<td>No</td>
</tr>
<tr>
<td>96–120</td>
<td>pMAL1a-8</td>
<td>No</td>
</tr>
<tr>
<td>120–145</td>
<td>pMAL1a-9</td>
<td>No</td>
</tr>
<tr>
<td>145–168</td>
<td>pMAL1a-10</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 6. Epitope mapping of monoclonal antibody mAB/B. Various regions of the CMV la protein (left) were expressed as fusions with the E. coli maltose-binding protein using the pMAL vectors indicated. The ability of mAB/B to interact with each fusion protein was assessed by immunoblotting. The methyltransferase-like (Rozanov et al., 1992) and helicase-like (Habili & Symons, 1989) domains are shown within the la protein as striped areas with backwards and forwards sloping lines respectively.

coli. The purified fusion protein retained the ability to bind to monoclonal antibodies mAB/2a3 and mAB/C in immunoblots. Epitope mapping of mAB/2a3 was carried out by making fusions of the maltose-binding protein with parts of the 2a protein using the pMAL-cRI vector and assaying their ability to bind to the antibody in immunoblots (Fig. 2). All the fusion proteins were detected when the immunoblots were probed with a polyclonal antiserum to the full-length 2a protein (Fig. 2a), but only some of them were detected with mAB/2a3 (Fig. 2b). The results, summarized in Fig. 3, indicated that the epitope in the 2a protein that interacted with mAB/2a3 lies between amino acids 599 and 620. This region contains the GDD motif (sequence motif VI, Koonin, 1991).

Evidence that binding to the region containing the GDD motif was responsible for the ability of mAB/2a3 to inhibit the CMV RdRp was obtained by incubating mAB/2a3 with the various fusion proteins, prior to addition to the RdRp reaction. The results (Fig. 4) showed that interaction of fusion proteins containing the GDD motif with mAB/2a3 largely prevented the antibody-mediated inhibition of the RdRp, whereas the fusion proteins lacking the GDD motif had little effect.

The antibody-mediated inhibition of the RdRp was also largely prevented by a peptide containing amino acids 601 to 612 of the 2a protein including the GDD motif, whereas another peptide of similar hydrophobicity had little effect (Fig. 5).

Epitope mapping of mAB/C was carried out by a similar method to that of mAB/2a3. This monoclonal antibody was also found to bind to a region of the 2a protein containing the GDD motif. Although the binding regions of mAB/C and mAB/2a3 were not distinguished at the level of resolution employed here, it is unlikely that both monoclonal antibodies bind to the same epitopes, since mAB/3 was a more effective RdRp inhibitor than mAB/C (Table 1).

Epitope mapping of inhibitory antibodies that bind to the 1a protein

The gene for the full-length 1a protein was cloned into the vector pMAL-cRI and fusion protein of the maltose-binding protein and the 1a protein was produced in E. coli. The purified fusion protein retained the ability to bind to monoclonal antibodies mAB/1a1 and mAB/B in immunoblots. Epitope mapping of mAB/B was carried
were peptide 1, HVDGVAGCGKTTAIC (contains peptides were used to immunize rabbits. The peptides coupled to keyhole limpet haemocyanin and the coupled protein; part of helicase motif V, Habili & Symons, domains of many positive-strand RNA viruses were amino acids 711 to 723 of the la protein; part of helicase motif I, Habili & Symons, 1989); peptide 2, KTVHESQ- peptide 3, QRRTGDAFTYFGNTC (contains amino acids 919 to 940. This region overlaps with parts of the la protein using the pMAL-cRI vector and assaying their ability to bind to antibody in immunoblots. The results (Fig. 6) indicated that the epitope in the la protein that interacted with mAB/B lies between amino acids 145 and 168. This is immediately adjacent to a conserved amino acid sequence motif (motif Ia2; Rozanov et al., 1992) found in the methyltransferase-like domain of the ‘altovirus’ group which includes CMV.

Epitope mapping of mAB/1a1 was carried out in a similar way to that of mAB/B. This monoclonal antibody was found to bind to a region of the la protein between amino acids 919 to 940. This region overlaps part of a conserved amino acid sequence motif (motif V; Habili & Symons, 1989) found in the helicase-like domain of supergroup A and some supergroup C viruses (supergroups as defined by Habili & Symons, 1989).

**Inhibition of the CMV RdRp by polyclonal antibodies induced by peptides corresponding to conserved sequence motifs in the la and 2a proteins**

Peptides corresponding to conserved amino acid sequence motifs in the helicase-like and polymerase-like domains of many positive-strand RNA viruses were coupled to keyhole limpet haemocyanin and the coupled peptides were used to immunize rabbits. The peptides were: peptide 1, HVDGVAGCGKTTAIC (contains amino acids 711 to 723 of the la protein; part of helicase motif I, Habili & Symons, 1989); peptide 2, KTVHESQ-GISEDHC (contains amino acids 928 to 940 of the la protein; part of helicase motif V, Habili & Symons, 1989); peptide 3, QRRTGDAFTYFGNTC (contains amino acids 570 to 583 of the 2a protein; part of polymerase motif V, Koonin, 1991); peptide 4, HRLLFGDDSLAFSC (contains amino acids 601 to 613 of the 2a protein; corresponds to polymerase motif VI, Koonin, 1991). The antibodies produced were tested for their ability to inhibit the activity of purified CMV RNA RdRp (Fig. 7). The most effective antibodies were induced by peptide 4 which contains the GDD motif; these antibodies almost completely inhibited the RdRp. Control reactions showed that the inhibitory effects of the antibodies were not due to degradation of the RNA template by contaminating nuclease.

**Discussion**

We have shown that four monoclonal antibodies that inhibit the CMV RdRp bind to epitopes within the methyltransferase-like, helicase-like and polymerase-like domains of the la and 2a proteins. Polyclonal antibodies induced by peptides corresponding to conserved sequence motifs within the helicase-like and polymerase-like domains also inhibited the CMV RdRp. The CMV RdRp complex contains the la and 2a proteins, and probably several host proteins (Hayes & Buck, 1990a, 1993). The ability of antibodies to inhibit the RdRp complex indicates that the epitopes to which the antibodies bind are located within the three-dimensional structure of the complex at positions that are accessible to antibodies. These include the Koonin (1991) polymerase-like motifs V (peptide 3 polyclonal antibodies) and VI (monoclonal antibodies mAB/2a3 and mAB/C; peptide 4 polyclonal antibodies), Habili & Symons (1989) helicase-like motifs I (peptide 1 polyclonal antibodies) and V (monoclonal antibody mAB/B and peptide 2 polyclonal antibodies) and a region immediately adjacent to Rozanov et al. (1992) methyltransferase-like domain Ia2 (monoclonal antibody mAB/B).

The most inhibitory antibodies were those directed against the Koonin (1991) polymerase-like motif VI (GDD motif). This motif is highly conserved in RNA-dependent RNA polymerases and also have counterparts in RNA-dependent DNA polymerases and DNA-dependent DNA polymerases (Delarue et al., 1990). The D residues of this motif have been postulated to be part of the active site of the enzyme and may bind the Mg$^{2+}$ ions required for catalysis of the nucleophilic attack of the 3'-OH of the primer on the α-phosphate of the incoming nucleotide triphosphate substrate (Argos, 1988; Delarue et al., 1990). The three-dimensional structures of he Klenow fragment of E. coli DNA polymerase I (Ollis et al., 1985), bacteriophage T7 RNA polymerase (Sousa et al., 1993) and human immunodeficiency virus type 1 reverse transcriptase (Kohlstaedt et al., 1992; Jacoba-Molina et al., 1993) indicate that the nucleic acid
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template-primer binding region lies within a cleft formed between ‘fingers’, ‘palm’ and ‘thumb’ subdomains of the polymerase. Sequence motifs equivalent to VI (Koonin, 1991) are located in the polymerase active site on the floor of the cleft within the palm subdomain, close to the 3'-OH of the primer strand. Delarue et al. (1990) have suggested that the basic structure of all polymerases may be similar. Hence the inhibitory effect of antibodies that bind to sequence motif VI could be due to their ability to interfere with the correct positioning of the template-primer at the catalytically active site of the CMV replicase.

Inhibition of the CMV RdRp activity by antibodies that bind to regions within the helicase domain of the 1a protein could be due to inhibition of the putative helicase activity of that protein. Helicase activity is likely to be needed to unwind the RNA duplex formed by the polymerase action, allowing multiple rounds of replication to occur. Helicase motif I (Habili & Symons, 1989) contains the consensus sequence G/AAXXXGK-(S/T) which is found in a wide variety of ATP-dependent helicases and other NTP-binding proteins (Walker et al., 1982; Gorbalenya et al., 1989, 1990; Gorbalenya & Koonin, 1989) and which in several cases has been shown to bind to the triphosphate moiety of the NTP (see, for example: Pai et al., 1990; Berchtold et al., 1993; Noel et al., 1993). Hence polyclonal antibodies to peptide 1 (which contains this sequence) might interfere with ATP binding. However, the inhibitory effect of these antibodies was relatively small. The function of helicase motif V, which corresponds to peptide 2 and is contained within the region to which monoclonal antibody mAB/1a1 mapped, is not known. Further studies will be needed to confirm that the 1a protein has the ATP-binding, ATPase and helicase activities suggested by its conserved sequence motifs and to determine whether or not the antibodies which bind to the helicase-like domain inhibit any of these activities.

Kao & Ahlquist (1992) have shown that the 1a and 2a proteins of brome mosaic virus (BMV) interact to form a stable complex. The region of the 1a protein involved in this interaction mapped to its helicase-like domain. As CMV has a similar genome organization to that of BMV, it is likely that the 1a and 2a proteins of CMV also interact in a similar manner. The ability of antibodies to bind to regions of the helicase-like domain of the CMV 1a protein within the replicase complex suggests that these regions are not involved in interaction with the 2a protein. Hence the helicase-like domain may contain different subdomains with different function.

The inhibition of the CMV RdRp by monoclonal antibody mAB/B which mapped to the methyltransferase-like domain of the 1a protein is interesting. It is likely that the 1a protein and equivalent proteins of other viruses are required for capping of the 5' end of the RNA. Capping requires nucleic acid triphosphatase, guanylyltransferase and methyltransferase activities. In Sindbis virus (SV) the nsP1 protein, which contains the methyltransferase-like domain, has been shown to have methyltransferase activity (Mi & Stollar, 1991) and there is evidence that replication of SV in vivo requires capping (Scheidel et al., 1989; Mi & Stollar, 1991). However, whether this requirement reflects a need for efficient translation of the virus RNA or for replication of the virus RNA per se is not known. Interference with methyltransferase activity cannot explain the inhibition of the CMV RdRp by mAB/B because the replication of CMV RNA can take place in the absence of S-adenosylmethionine, the methyl donor for methylation, which was not present in our RdRp assays (Hayes & Buck, 1990a, 1993), although an effect on either of the other two enzymes required for capping cannot be excluded. SV mutants which are defective in the initiation of minus-strand synthesis map to the nsP1 protein, but outside the methyltransferase-like domain (Hahn et al., 1989). Further work will be needed to define the enzymic activities of the CMV 1a protein and which of these are inhibited by mAB/B.

Although the inhibitory effects of the antibodies were probably due to direct effects on the functions of the domains to which the antibodies bound as described above, the possibility that antibody binding might perturb the structure of the replicase complex and cause inhibition as a secondary effect cannot be eliminated.

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References


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