Translation of the RNA of Cowpea Severe Mosaic Virus in vitro and in Cowpea Protoplasts

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SUMMARY

The multiplication of the DG strain of cowpea severe mosaic virus (CPsMV-DG) was studied in cowpea protoplasts prepared from infected leaves and in protoplasts inoculated in vitro. Up to six proteins were detected in DG-infected cells which were either absent in mock-infected protoplasts or present in smaller amounts. Their mol. wt. were estimated to be 125000, 98000, 86000, 65000, 39000 and 22000. The latter two probably represent the two viral capsid proteins. The 125000 mol. wt. protein was found early in infection and was synthesized at a high rate throughout infection. It was detected, together with the 86000 mol. wt. protein, in protoplasts that had been inoculated with purified bottom component alone.

CPsMV was also translated in a messenger-dependent reticulocyte lysate. Total DG strain RNA directed the synthesis of polypeptides with mol. wt. of 200000, 125000, 108000, 98000 and 42000. No cleavage of the in vitro products was observed by varying the temperature or the concentration of dithiothreitol (DTT) in the translation mixture. The polypeptides induced by CPsMV-DG were compared with those induced by the SB strain of cowpea mosaic virus. Since the 170000 and 30000 mol. wt. proteins typical of the SB strain were not detected among the DG-specific proteins either in vivo or in vitro, a different processing of the large precursor of 200000 mol. wt. seems to take place.

INTRODUCTION

Members of the comovirus group comprise two components, middle (M) and bottom (B), which are both necessary for infection. The SB strain of cowpea mosaic virus (CPMV-SB) is often regarded as the type member of this group and most of the research has been carried out with this particular virus. Each component of CPMV-SB contains one RNA species of mol. wt. 1.37 × 10^6 (M-RNA) and 2.02 × 10^6 (B-RNA) respectively (Reijnders et al., 1974). These RNAs have a poly(A) sequence at the 3'-end (El Manna & Bruening, 1973; Steele & Frist, 1978) and a small protein attached to their 5'-terminus (Stanley et al., 1978; Daubert et al., 1978).

CPMV can multiply to a high level in in vitro inoculated cowpea protoplasts (Beier & Bruening, 1975; Hibi et al., 1975). At least seven virus-specific proteins were detected in infected protoplasts, two of which were identified as the viral capsid proteins (Rottier et al., 1979; Rezelman et al., 1980). Little is known about the function of the other virus proteins. Among them, a prominent polypeptide of 170000 mol. wt. and a protein of 30000 mol. wt. were visible early in infection after inoculation with the SB strain of CPMV and apparently are encoded by the B-RNA. They were detected in cowpea protoplasts inoculated with purified bottom component only and they were synthesized in vitro under the direction of B-RNA (Rezelman et al., 1980).
In cell-free systems a polypeptide of 205,000 mol. wt. and two polypeptides of 108,000 and 96,000 mol. wt. were directed by B-RNA and M-RNA respectively (Davies et al., 1977; Pelham, 1979). Cleavage of the large B-RNA product to 170,000 and 30,000 mol. wt. required ATP, a reticulocyte factor and the presence of dithiothreitol (DTT). Cleavage of the M-RNA products occurred only when cleaved B-RNA proteins were present. Among the latter, the polypeptide of 30,000 mol. wt. is suggested to be a virus-specific protease responsible for the cleavage of the large precursor molecules (Pelham, 1979).

We have studied the \textit{in vivo} and \textit{in vitro} synthesized polypeptides from another comovirus, the DG strain of cowpea severe mosaic virus (CPsMV). CPsMV was formerly called a severe strain or subgroup of cowpea mosaic virus (CPMV) (Agrawal, 1964) but has recently been treated as a distinct virus (De Jager, 1979). It differs from the SB strain of CPMV in host range, antigenic properties, lack of complementation between heterologous components and its high M/B component ratio (Swaans & van Kammen, 1973).

In view of the reported differences it was interesting to compare the virus-specific proteins synthesized by the two viruses. Proteins essential for virus growth might well have been conserved during evolution in all comoviruses and altered only slightly in their amino acid composition and/or mol. wt. Other proteins might have undergone extensive changes and thus contribute to different responses of a host to infection with related viruses.

Hence, we compared the protein patterns of SB and DG in different hosts using protoplasts and leaf discs as cell systems. Furthermore, we also translated CPsMV RNA in a messenger-dependent reticulocyte lysate.

\section*{METHODS}
\textbf{Viruses and plant varieties.} The SB isolate of CPMV was from Dr G. Bruening (University of California, Davis). The origin of the DG strain of CPsMV and the varieties of cowpea \textit{(Vigna sinensis, cv. Blackeye 5 and Chinese red × Iron)} have been described previously (Beier et al., 1977). For brevity, the two viruses will be denoted SB and DG respectively. Both viruses were propagated in Blackeye 5 cowpeas and were isolated in 50 mM-potassium phosphate, 2 mM-EDTA pH 7 according to the large scale procedure of Bruening (1969). RNA was obtained from purified virus by phenol extraction and subsequent ethanol precipitation. The RNAs were analysed for their integrity in sucrose gradients and polyacrylamide gels. Concentrations were estimated spectrophotometrically by using an absorption coefficient of $A^{0.1\%}_{260} \approx 22$.

\textbf{Isolation and characterization of virus components.} Components of CPsMV-DG were isolated by two successive equilibrium gradient centrifugations in CsCl (Ti50 rotor for 36 h at 45,000 rev/min). The initial CsCl solution had a density of 1.41 g/ml. The banded material was recovered by puncturing the centrifuge tube with a needle. As with CPMV-SB (Bruening, 1969) two forms of the bottom component were found in the density gradient. One had a buoyant density only slightly greater than that of M (B\textsubscript{U}), whereas the other was found at equilibrium near the bottom of the centrifuge tube (B\textsubscript{L}). Either B\textsubscript{U} or B\textsubscript{L} could serve with M to initiate infections. For all experiments, we used only B\textsubscript{L}-component fractions of CPsMV because of their low contamination by M. Concentrations of M component, B\textsubscript{L} component and unfractionated virus were estimated spectrophotometrically using $A^{0.1\%}_{260} = 6.8, 9.5 \text{ and } 8$ respectively. Mixtures of M and B components were more than 60 times as infectious as the single components alone. Residual infectivity of M and B\textsubscript{L} components were 0.25 and 0.17 lesions per leaf of cowpea \textit{(V. sinensis cv. Arlington)} at a concentration of $2 \times 10^{11}$ particles/ml respectively.

\textbf{Isolation and inoculation of protoplasts.} Cowpea protoplasts were isolated from the
primary leaves of 8-day-old cowpeas as described previously (Beier & Bruening, 1976). For infection, purified CPMV preparations were dissolved to a concentration of 10 µg/ml in 0-1 M-potassium phosphate buffer pH 6 containing 0-45 M-mannitol and 75 µg/ml protamine sulphate (Calbiochem). The solution was preincubated at 25 °C for 5 min. Protoplasts were suspended directly in the virus solution and incubated for 15 min. Washed protoplasts were cultivated in the medium used by Aoki & Takebe (1969) except that 6-benzyladenine and 2,4-dichlorophenoxyacetic acid were omitted. Gentamicin (Sigma) and mycostatin (Serva) were added at final concentrations of 20 µg/ml and 15 µg/ml respectively. Protoplasts were incubated in 5 ml portions in Petri dishes at 26 °C under continuous illumination (approx. 2000 lux). Extracts of protoplasts were prepared for virus assay as described by Beier & Bruening (1975). Infectivity assays of CPMV-SB and CPsMV-DG were performed on Chinese red x Iron and Arlington cowpeas respectively.

Incorporation of radioactive precursors. For incorporation studies protoplasts were cultivated in small Petri dishes (diam. 3 cm) in 3 ml Aoki medium (Aoki & Takebe, 1969) at concentrations of about 5 x 10^5 cells/ml. Unless otherwise stated 2 µCi/ml 35S-methionine (1000 Ci/mmol) was added to the protoplasts 15 to 18 h after inoculation. After appropriate periods of incubation the protoplasts from a 200 µl sample were collected by sedimentation in an Eppendorf centrifuge, suspended in 100 µl of hot sample buffer containing 0-05 M-tris-HCl pH 6-8, 2 % SDS, 2-5 % β-mercaptoethanol and 10 % glycerol, heated at 95 °C for 3 min and stored at −20 °C. Twenty µl amounts were used for gel electrophoresis.

To study protein synthesis in situ 7-day-old primary leaves were inoculated with the SB or DG strains at concentrations of 75 µg/ml and 15 µg/ml respectively; control plants were rubbed with 0-1 M-potassium phosphate buffer pH 7. Carborundum was used as an abrasive in all mechanical inoculations. Two h later leaves were removed and cut to fit in a small Petri dish (diam. 2-5 cm). The lower side of the leaf discs was brushed lightly with carborundum and then floated on 2 ml Aoki culture medium from which mannitol was omitted. About 18 h later the medium was replaced by fresh medium to which 35S-methionine (15 µCi/ml) had been added. The Petri dishes were placed in a growth chamber under 18 h of dark illumination (about 8000 lux). The day and night temperatures were 27 °C and 23 °C respectively. At appropriate times protoplasts were isolated from the leaf discs and prepared for gel electrophoresis as described earlier.

In vitro protein syntheses. In vitro translation of CPMV RNAs was performed in a messenger-dependent reticulocyte lysate according to Pelham & Jackson (1976) except that no unlabelled amino acids were added. For the translation of viral RNAs 80 µl of micrococcal nuclease-treated lysate was supplemented with 2 µl 0-2 M-DTT, 10 µl of 10 mg/ml calf liver tRNA (Boehringer, Mannheim) and 7-5 µl 35S-methionine (7 mCi/ml, 1250 Ci/mmol). A 9 µl amount was used per assay to which 1 µl CPMV RNA (500 µg/ml) was added. The reaction was stopped after incubation for 60 min at 30 °C by adding 10 µl of hot sample buffer (Laemmli, 1970) to each assay tube. Samples were stored frozen at −20 °C. Before electrophoresis samples were thawed and heated for 3 min at 95 °C.

Polyacrylamide slab-gel electrophoresis. Proteins were analysed by electrophoresis in either 8 or 10 % polyacrylamide gel slabs containing SDS (Laemmli, 1970; Issinger & Falk, 1976). Gels were stained (0-25 % Coomassie Brilliant Blue R-250 in 50 % ethanol and 10 % acetic acid), destained and then processed for fluorography (Laskey & Mills, 1975) or autoradiography. Exposure of the X-ray films (Kodak RP Royal X-Omat) was for 2 days or 2 to 3 weeks to detect proteins labelled in vitro and in vivo respectively. Mol. wt. were estimated relative to Escherichia coli RNA polymerase (165000, 155000), β-galactosidase (116000), phosphorylase a (94000), bovine serum albumin (68000), fumarase (49000), carbonic anhydrase (28000) and tobacco mosaic virus (TMV) coat protein (17500).
H. BEIER AND OTHERS

Fig. 1. Virus accumulation in cowpea protoplasts following inoculation of the isolated protoplasts or intact leaves. (a) Freshly isolated Chinese red × Iron cowpea protoplasts were inoculated with either CPMV-SB (○) or CPsMV-DG (△) at 10 μg/ml. Aliquots of the protoplast suspensions were taken for preparation of extracts and local lesion assay at the times indicated. Virion particle counts were made by comparison of local lesion counts with those given by known concentrations of purified virus. (b) Primary leaves of 7-day-old cowpeas were inoculated with the SB (○) and DG (△) strains at concentrations of 75 μg/ml and 15 μg/ml respectively. The abscissa records the time interval between inoculation of the primary leaves and the onset of protoplast isolation from equal weights of leaves. The protoplast suspensions were disrupted and assayed on local lesion hosts as described.

RESULTS

Replication of CPsMV-DG and CPMV-SB in cowpea protoplasts prepared from infected leaves and in protoplasts inoculated in vitro

We compared the replication of SB and DG strains in two cowpea lines, Chinese red × Iron being a hypersensitive local lesion host for the SB and a systemic host for the DG strain, and Blackeye 5 cowpea being susceptible to both viruses (Beier et al., 1979). If Chinese cowpea protoplasts were inoculated in vitro, the growth curves for SB and DG strains were very similar (Fig. 1a) and could be superimposed on growth curves of the two viruses in Blackeye cowpea protoplasts. Since the hypersensitive reaction was not expressed in Chinese cowpea protoplasts infected with SB, we inoculated intact leaves with virus, and isolated protoplasts at various times after inoculation (Fig. 1b). The hypersensitive reaction then seemed to influence the replication of SB. An increase in protoplast-associated virus was observed over the period 15 to 40 h after inoculation which was comparable to the steep rise in the number of virus particles per protoplast following inoculation of Chinese cowpea leaves with DG (Fig. 1b) or Blackeye cowpeas with either of the viruses (data not shown). Moreover, during this time interval (15 to 40 h) the percentage of viable protoplasts and the number of protoplasts per g leaf tissue were about the same if they were isolated from systemic or hypersensitive reacting cowpea lines. Thus, virus accumulation was sufficient to study viral protein synthesis in all virus–host systems at least up to 40 h post-inoculation. Later on, protoplast-associated virus declined rapidly in leaf tissue from Chinese cowpeas infected with SB (Fig. 1b). At the same time the yield of protoplasts per leaf was reduced but the percentage of viable protoplasts remained high (about 80%). Necrotic local lesions became visible as early as 40 h post-inoculation if leaves were heated in a boiling water bath for 2 min and decolourized with 95% ethanol.

When the replication of SB was followed by staining protoplasts from infected leaves with fluorescein isothiocyanate (FITC)-conjugated antibodies, we found that 40 h after inoculation only 1 to 2% of the living protoplasts derived from infected Chinese cowpea leaves were
**CPSMV protein synthesis in vivo and in vitro**

Electrophoretic analysis of $^{35}$S-labelled proteins synthesized in Chinese red × Iron cowpea protoplasts inoculated with CPMV-SB or CPSMV-DG. Inoculation of isolated protoplasts with virus was as described in Methods; mock inoculation (−) was with buffer only. Protoplasts were incubated for the times indicated and $^{35}$S-methionine (2 μCi/ml) was added 17 h after inoculation. Protein samples were analysed on a 10% SDS–polyacrylamide gel. Numbers on the left and right indicate mol. wt. ($\times 10^{-3}$) of SB- and DG-induced proteins respectively. The capsid proteins of SB and DG stained with Coomassie Blue are shown adjacent to the autoradiogram.

![Electrophoretic analysis](image)

**Fig. 2.** Electrophoretic analysis of $^{35}$S-labelled proteins synthesized in Chinese red × Iron cowpea protoplasts inoculated with CPMV-SB or CPSMV-DG. Inoculation of isolated protoplasts with virus was as described in Methods; mock inoculation (−) was with buffer only. Protoplasts were incubated for the times indicated and $^{35}$S-methionine (2 μCi/ml) was added 17 h after inoculation. Protein samples were analysed on a 10% SDS–polyacrylamide gel. Numbers on the left and right indicate mol. wt. ($\times 10^{-3}$) of SB- and DG-induced proteins respectively. The capsid proteins of SB and DG stained with Coomassie Blue are shown adjacent to the autoradiogram.

Thus, comparison of the infectivity assay and results obtained by the immunofluorescence technique gave somewhat inconsistent results.

**Virus protein synthesis in protoplasts inoculated in vitro**

Electrophoretic analysis of the proteins synthesized in in vitro inoculated Chinese cowpea protoplasts is shown in Fig. 2. Four proteins of mol. wt. 125 000, 86 000, 39 000 and 22 000, absent in mock-infected cells, were detected in protoplasts inoculated with CPSMV-DG and four proteins of mol. wt. 170 000, 38 000, 30 000 and 24 000 were observed in protoplasts infected with CPMV-SB. In addition, the synthesis of two proteins of 86 000 and 64 000 appeared to be enhanced in SB-infected cells. Proteins identified as specific for SB in Chinese cowpea protoplasts are of similar size, as previously shown for the proteins synthesized in SB-infected Blackeye cowpea protoplasts (Rottier et al., 1979; Rezelman et al., 1980). These authors detected eight virus-specific proteins of mol. wt. 170 000, 110 000, 87 000, 84 000, 60 000, 37 000, 32 000 and 23 000.

The 38 000 and 24 000 mol. wt. proteins specific for SB and the 39 000 and 22 000 mol. wt. proteins specific for DG probably represent the viral capsid proteins. We found two species of
Fig. 3. Electrophoretic analysis of $^{35}$S-labelled proteins synthesized in Blackeye cowpea protoplasts inoculated with CPsMV-DG components. Protoplasts were either mock-infected (−), inoculated with purified middle (M) or bottom (B) components or inoculated with complete virus (M + B) at concentrations of 10 µg/ml. Protoplasts were incubated for 70 h and $^{35}$S-methionine (3 μCi/ml) was added 20 h after inoculation. Protein samples were analysed on a 10% SDS-polyacrylamide gel which was then subjected to autoradiography.

the smaller coat protein deriving from DG virions with mol. wt. of 22 000 and 20 200, and up to four S protein species in the protein pattern of SB particles (Fig. 2). In protoplasts, however, we observed only one S protein similar in size to the slow migrating species of the S protein of SB and DG respectively.

A major difference between the protein patterns of SB and DG was the synthesis of a 170 000 mol. wt. protein in SB-infected and a 125 000 mol. wt. protein in DG-infected protoplasts. The 125 000 mol. wt. polypeptide had some features which were also characteristic of the 170 000 mol. wt. protein. It was already present as early as 15 h after inoculation and was synthesized at a high rate throughout infection (Fig. 2).

The 125 000 mol. wt. protein, in addition to the 86 000 mol. wt. protein, appeared to be coded by the bottom component of DG since both proteins were found in protoplasts inoculated with purified bottom component only. No virus-specific proteins were detected upon inoculation of protoplasts with purified middle component of DG (Fig. 3).

Virus protein synthesis in protoplasts prepared from infected leaves

If leaves from Chinese cowpeas were detached after inoculation with SB or DG and floated on a medium containing inorganic salts (Aoki medium) or simply 0·1 M-potassium phosphate buffer pH 7, the number of necrotic and chlorotic local lesions was the same compared to intact plants, as was the time at which lesions first became visible. The uptake of
Fig. 4. Electrophoretic analysis of ³⁵S-labelled proteins synthesized in cowpea protoplasts prepared from infected leaves. Primary leaves of (a) Chinese red x Iron or (b) Blackeye 5 cowpeas were inoculated with CPMV-SB, CPsMV-DG or buffer. Leaf discs were floated on a medium containing ³⁵S-methionine as described in Methods. Protoplasts were isolated from the leaf discs 42 h post-inoculation and aliquots were used for protein analysis on either 8 % (right) or 10% (left) gels.
Fig. 5. Sucrose gradient analysis of RNA preparations of CPMV-SB and CPsMV-DG. RNA was obtained from purified virus by phenol extraction and subsequent ethanol precipitation. A 15 μg amount of each viral RNA was applied to a 5 to 20% (w/v) linear sucrose gradient and sedimented in a Beckman SW56 rotor for 2.5 h at 48,000 rev/min. Gradients were monitored from the top using an ISCO apparatus.

35S-methionine into leaf discs was as rapid and efficient as the uptake of this amino acid by isolated protoplasts. All virus-specific proteins visible 42 h after inoculation of leaves (Fig. 4) were detected at 20 h post-inoculation and after a 3 h labelling period with 35S-methionine.

Virus proteins could be distinguished from the background of host proteins more readily in protoplasts prepared from infected plants than in in vitro inoculated protoplasts. For instance, a protein of mol. wt. 98,000, not previously identified as DG-specific in infected protoplasts, was synthesized at higher rates than a host protein of similar size in DG-infected leaf discs (Fig. 4a, b). Siegel et al. (1978) have reported that virus protein synthesis occurs in addition to, rather than at the expense of, normal cellular protein synthesis in TMV-infected protoplasts. It is possible that in infected leaves host protein synthesis does not proceed at its normal rate and/or that virus accumulation reaches a higher level. On the other hand, a general activation of host genes might take place upon incubation of protoplasts, caused by the isolation procedure and the high osmolarity of the culture medium.

No virus proteins could be detected in Chinese cowpea protoplasts isolated from leaves 42 h after inoculation with CPMV-SB (Fig. 4a). If the radioactive amino acid was added to the leaf discs 2 h after inoculation or if the concentration of SB in the inoculum was reduced to 15 or 7.5 μg/ml, the effect on protein synthesis was the same. This was unexpected since protoplasts derived from the hypersensitive host were found to produce infectious material up to 40 h post-inoculation as mentioned earlier (Fig. 1b). On the other hand, only small amounts of antigen were discovered by staining with fluorescent antibody. Possibly the observed rise of protoplast-associated virus as determined by local lesion assay was in part due to viral RNA replication rather than to an increase in infectious particles. This is explicable if one assumes that the action of host nucleases was reduced by the precautions we took during the preparation of extracts (e.g. quick homogenization of the protoplasts in the cold).
CPsMV protein synthesis in vivo and in vitro

Fig. 6. Electrophoretic analysis of the in vitro translation products directed by CPMV RNAs at different temperatures. The reaction was performed in the absence of DTT (a, b) or in the presence of 4 mM-DTT (c, d, e). Incubation was for 60 min at 30 °C (a, c, e) or 37 °C (b, d). The reaction mixture contained 50 μg/ml SB-RNA (a, b), DG-RNA (d, e) or no RNA (c). Products were analysed on a 10% polyacrylamide gel which was then subjected to autoradiography.

Fig. 7. Comparison of the CPsMV-DG proteins induced in cowpea protoplasts with the in vitro translation products directed by DG-RNA. Blackeye cowpea protoplasts were inoculated with DG (+) or buffer (−). Protoplasts were incubated for 70 h and 35S-methionine was added to the culture medium 18 h after inoculation. The viral RNA was translated in a messenger-dependent reticulocyte lysate. The reaction mixture contained 35S-methionine (500 μCi/ml), 4 mM-DTT, 1 mg/ml tRNA and 50 μg/ml DG-RNA. Samples were analysed on a 10% polyacrylamide gel which was then fluorographed. Numbers on the left and right indicate mol. wt. (×10^{-3}) of the in vivo and in vitro CPsMV-specific proteins respectively.

In vitro translation of DG-RNA in a messenger-dependent reticulocyte lysate

A sucrose gradient analysis of the viral RNAs used for the in vitro translation experiments is shown in Fig. 5. The ratio of B/M component differed in SB and DG ribonucleoprotein particles and their isolated RNAs. The percentage of B component in SB preparations varied between 60 and 70% compared to 10 to 20% in DG preparations. Different B/M ratios for different comoviruses have been reported by other workers (van Kammen, 1968; Thongmeearkom & Goodman, 1978).

Translation of DG-RNA in vitro resulted in the synthesis of three major proteins of 108000, 98000 and 42000 mol. wt. and among others two minor proteins of 200000 and 125000 mol. wt. (Fig. 6e). The in vitro translation products directed by DG-RNA were
compared with the in vitro synthesized polypeptides of the RNA of CPMV-SB. It was confirmed that SB coded in vitro for five major polypeptides with mol. wt. of 200000, 170000, 110000, 98000 and 30000 (Fig. 6a).

The in vitro translation of DG-RNA was unaffected by higher temperatures (although the overall synthesis rate was reduced) and no cleavage of any polypeptide was stimulated by adding DTT to the translation mixture (Fig. 6d, e). In contrast, cleavage of the 200000 mol. wt. protein translated from SB-RNA was prevented at 37 °C (Fig. 6b), but was not dependent on the presence of DTT as demonstrated by Pelham (1979).

The 98000 and 110000 mol. wt. proteins are precursor polypeptides of overlapping sequences accounting for about 80% of the coding capacity of the M-RNA of CPMV-SB (Davies et al., 1977; Pelham, 1979). Two polypeptides of similar mol. wt. were synthesized from the total RNA of DG (Fig. 6e) and they possibly originate also from M-RNA. Proteolytic cleavage of these precursors seems unlikely to have occurred in the in vitro assay since sequential appearance of proteins of intermediate size was not observed in time course experiments (data not shown).

Comparison of the in vitro polypeptides with proteins found in DG-infected cowpea protoplasts showed that some are of similar size (Fig. 7). The 125000 mol. wt. protein synthesized in large amounts in vivo was only a minor protein among the in vitro translation products and its identity with the in vivo protein has to be established. The reduced synthesis of it in vitro probably reflects the low percentage of B-RNA (about 15%) present in unfractionated RNA preparations (Fig. 5).

The synthesis of a protein of 98000 mol. wt. was enhanced in DG-infected protoplasts or leaf discs (Fig. 4 and 7) and this protein might be identical with the corresponding protein seen in vitro. No polypeptide of 108000 mol. wt. was found in DG-infected cells. It may be that the initiation site for the 98000 mol. wt. protein is more efficiently translated in vitro than the initiation site for the 108000 mol. wt. protein. The origin of the 42000 mol. wt. polypeptide translated from DG-RNA remains obscure since a protein of similar size was not discovered in infected protoplasts. Endogenous protein synthesis can be excluded (Fig. 6d). No viral coat proteins of the size 39000 and 22000 mol. wt. were translated from DG-RNA in vitro (Fig. 7).

DISCUSSION

Up to six proteins with mol. wt. of 125000, 98000, 86000, 65000, 39000 and 22000 were detected in protoplasts or leaf discs infected with the DG strain of CPsMV. Of these, all but the 125000 mol. wt. protein are of similar size to proteins encoded by the SB strain of CPMV (Rottier et al., 1979; Rezelman et al., 1980).

The 39000 and 22000 mol. wt. proteins were identified as the two viral capsid proteins by comparing their electrophoretic mobility in SDS–polyacrylamide gels with those of disrupted virions. As with CPMV-SB (Davies et al., 1977; Pelham, 1979) they were not translated in vitro from total DG-RNA. The reason for this is not known but might be due to the need of specific host enzymes or cytological structures absent in the reticulocyte lysate.

The polypeptide of 125000 mol. wt. shares some features with the 170000 mol. wt. protein, the latter being one of the major proteins produced in SB-infected cells (Rottier et al., 1979). Both proteins are detected early in infection and are synthesized in large amounts throughout infection. Moreover, the 125000 mol. wt. protein is very likely coded by the bottom component since it was found in cowpea protoplasts inoculated with purified bottom component only. The same has been shown for the 170000 mol. wt. protein (Goldbach et al., 1980; Rezelman et al., 1980). Whether the 125000 mol. wt. polypeptide arises by cleavage from a large precursor molecule is not clear. Although a polypeptide of about 200000 mol. wt. was formed in vitro from DG-RNA, cleavage of the latter was not observed. Factors
which stimulated the primary cleavage of the SB polypeptide did not seem to have any effect on the relative amounts of the large DG polypeptides (Fig. 6). Alternatively, the synthesis of the 125000 mol. wt. protein may be initiated at an internal initiation site on the B-RNA of DG which could explain its high synthesis rate in vivo. Evidence for internal initiation sites on viral RNAs has been presented for picornaviruses (Jense et al., 1978) and recently for the M-RNA of CPMV-SB (Pelham, 1979).

As well as the 125000 mol. wt. polypeptide, the 86000 mol. wt. protein specific for DG is also coded by the B-RNA, as is the corresponding protein in SB-infected protoplasts (Rottier, 1980; Goldbach et al., 1980). By comparison of their proteolytic digestion patterns it has been shown that nearly all peptides of the 87000 mol. wt. polypeptide could be recovered in the 170000 mol. wt. peptide maps, suggesting that the 87000 mol. wt. protein arises by cleavage from the latter (Rezelman et al., 1980). Since there is no 170000 mol. wt. protein synthesized in DG-infected cells, the 86000 mol. wt. polypeptide specific for DG must originate either by cleavage from the 200000 mol. wt. precursor molecule or by processing of the 125000 mol. wt. protein.

So far, it appears as if the precursors translated from B- and M-RNA are about the same size for SB and DG if one assumes the polypeptides of 200000, 108000 and 98000 mol. wt. translated from total DG-RNA to be the counterparts of the SB polypeptides. However, processing of the large molecules to virus-specific end-products seems to proceed by different successive cleavages in the two viruses.

A protein of 30000 mol. wt. typical for CPMV-SB was not found in DG-infected cells or among the products translated in vitro from DG-RNA. The possibility that a 30000 mol. wt. protein was synthesized in DG-infected protoplasts at very low rates or that it co-migrated with a host protein of similar size cannot be ruled out completely. Yet in this case its rate of synthesis or its processing cannot be correlated with the 125000 mol. wt. protein, the latter being a major DG protein. Such a correlation was shown for the 170000 and 30000 mol. wt. polypeptides in infections with SB (Rezelman et al., 1980).

If there is really no de novo synthesis of a 30000 mol. wt. protein after infection with DG, this protein might be part of one of the other high mol. wt. polypeptides of still unknown functions. However, it might also completely lack any essential virus function for the DG strain of CPsMV.

A need for different enzyme activities would partly explain why heterologous mixtures of M and B components of SB and DG are not compatible (H. Beier et al., unpublished observations). Generally, it appears that strains of distantly related comoviruses cannot complement each other. For instance, no complementation takes place in heterologous mixtures of M and B from cowpea yellow mosaic virus and bean pod mottle virus (Wood & Bancroft, 1965) or in component mixtures from the Nigerian and Trinidad isolates of CPMV (van Kammen, 1968), but complementation occurs in heterologous mixtures of M and B from the Arkansas and Puerto Rico strains, the latter being both members of CPsMV (Thongmeearkom & Goodman, 1978).

It would be interesting to study the virus protein synthesis of other members of the comoviruses to find out whether all of those belonging to the same group have similar protein patterns.

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