Location of Structural Proteins in Particles of Rice Gall Dwarf Virus

By T. OMURA, 1* Y. MINOBE, 2 M. MATSUOKA, 2 Y. NOZU, 2 T. TSUCHIZAKI 1 AND Y. SAITO 3

1 National Agriculture Research Center, 2 National Institute of Agrobiological Resources and 3 National Institute of Agro-environmental Sciences, Tsukuba Science City, Ibaraki 305, Japan

(Accepted 19 December 1984)

SUMMARY

Protein from particles of rice gall dwarf virus was resolved into seven components by polyacrylamide gel electrophoresis. The estimated molecular weights (× 10^3) were 183, 165, 150, 143, 120, 56 and 45 (K). Cores prepared by treating intact particles with CsCl contained the 183K, 165K, 120K and 56K proteins; the 150K, 143K and 45K proteins were recovered from the top layer of gradients after CsCl equilibrium centrifugation. Antiserum against intact particles reacted mainly with 150K and 45K proteins and antiserum against core particles reacted mainly with 183K and 120K proteins. The results suggest that the 150K and 45K proteins are located on the surface of the outer capsid, that the 183K and 120K proteins are on the surface of the core, that the 165K and 56K proteins are inside the core, and that the 143K protein is either inside the outer capsid or inside the core.

INTRODUCTION

Rice gall dwarf virus (RGDV) is a recently described virus of rice (Omura et al., 1980; Ong & Omura, 1982; Faan et al., 1983) that is transmitted in a persistent manner by five species of leafhoppers (Inoue & Omura, 1982; Morinaka et al., 1982) and which has polyhedral particles about 65 nm in diameter. RGDV is thought to be a Phytoreovirus (Omura et al., 1982), a suggestion supported by the finding that the genome of RGDV consists of 12 segments of double-stranded RNA (Hibi et al., 1984).

Protein components of the virus particles have been studied in some viruses belonging to the reovirus group. The Phytoreoviruses such as wound tumour virus (WTV) (Reddy & Macleod, 1976) and rice dwarf virus (RDV) (Nakata et al., 1978) have six or seven similar-sized protein components that differ in size from the six particle proteins of maize rough dwarf virus (Boccardo & Milne, 1975) and the three particle proteins of core preparations of Fiji disease virus (Van der Lubbe et al., 1979), both of which are Fijiviruses. We have examined the number and molecular weights of the protein components of RGDV to see if these are similar to those of WTV and RDV. Also, outer capsids were removed from core particles by a CsCl treatment and the locations of the protein components were investigated by electrophoresis and immunoblotting using antisera to virus particles and to cores.

METHODS

Virus. RGDV was purified by the method reported by Omura et al. (1982), except that 1% Triton X-100 was added to the extract containing polyethylene glycol and sodium chloride. The final pellets were resuspended in 0.1 M-histidine, 0.01 M-MgCl2, adjusted pH to 7.0 with HCl (His-Mg) and stored at −70°C until use.

CsCl equilibrium centrifugation. A purified preparation of RGDV suspended in 0.1 ml His-Mg was added to a solution of CsCl to make the final concentration of CsCl 36% (w/w). The mixture was kept for 40 min at room temperature (approx. 20°C) and centrifuged at 165000 g for 24 h at 4°C in a Hitachi RP-50 rotor. The tube contents were fractionated with an ISCO model UA5 ultraviolet analyser and virus particles or core particles were recovered by centrifugation (96000 g) for 2 h at 4°C in a Hitachi RP-40 rotor and resuspended in His-Mg. The density of the fractions was calculated from their refractive indices using the method of Brakke (1967).
The fraction from the meniscus to about 3 mm below the meniscus was mixed with 0.2 vol. 50% trichloroacetic acid and centrifuged. The pellet was washed twice with acetone, then dried and dissolved in His-Mg containing 2% SDS and 2% 2-mercaptoethanol. The sample was frozen at -20°C until use.

Electron microscopy. Preparations were negatively stained with either 2% neutralized phosphotungstic acid (PTA) or 2% uranyl acetate and were examined in a Hitachi H-500 electron microscope.

Antisera. A rabbit was immunized by intramuscular injection of purified preparations of core particles emulsified with an equal volume of Freund's complete adjuvant followed by two similar intravaneous injections at 10-day intervals. The titre of the antiserum in a precipitin ring interface test was 1/64 against homologous inner core particles. Sera were stored at -70°C until use.

Electrophoresis. Slab gels consisting of 10% polyacrylamide (acrylamide : bisacrylamide = 30:0.8) containing 0.1% SDS were prepared by the method of Laemmli (1970). Purified materials were solubilized by heating for 2 min at 100°C in 2% SDS and 2% 2-mercaptoethanol. Molecular weights of proteins were determined using SDS-PAGE Marker I (molecular weights were 180, 140, 100, 42 and 39, all × 103; Biochemicals, Tokyo, Japan) as standards. Gels were stained with Coomassie Brilliant Blue R (CBB) and scanned using a Shimadzu chromatoscanner CS-910. The area under each absorbance peak was taken as a measure of the amount of protein in the band.

Immunoblotting. Polypeptides were transferred from polyacrylamide gels to nitrocellulose sheets using a Transfer Blotting Apparatus (Model ETB-15; Toyo Science, Japan) as described by Towbin et al. (1979). Transfer of the polypeptides to the nitrocellulose sheet was confirmed by staining with amido black. Immunological detection of polypeptides on nitrocellulose sheets using fluorescein-conjugated sheep anti-rabbit IgG (Cappel Laboratories) was performed by the method of Towbin et al. (1979) modified by Matsuoka & Asahi (1983).

RESULTS

Fractionation of virus particles using CsCl

Reddy & Macleod (1976) and Nakata et al. (1978) separated the outer capsid from the core of WTV and RDV, respectively, by centrifuging particles in CsCl density gradients; this method was applied to RGDV particles. The pH of the buffer has been reported to have a marked effect on CsCl equilibrium sedimentation of bluetongue virus (Verwoerd et al., 1972) and WTV (Reddy & Macleod, 1976). Therefore, we studied the effect of centrifuging RGDV particles in CsCl in His-Mg buffer at different pH values. The results are shown diagrammatically in Fig. 1. Particles that were penetrable by stains and that appeared empty were always found in a zone with a density of 1.285 g/ml. At pH 6.0 a broad band formed with a density of 1.406 g/ml. It was less obvious at higher pH and was not observed at pH > 7.5. This band contained virus particles from which part of the outer capsid had been lost. At pH > 6, a clear band appeared which had a density of 1.496 g/ml and which contained polyhedral particles with spike-like structures about 50 nm in diameter (Fig. 2, right). These particles were designated as core particles. From these results, it appears that the outer capsid proteins were removed by CsCl when the pH was higher than 7.5 and His-Mg-buffered CsCl, pH 7.5, was adopted to separate the outer capsid proteins from the core particles.

The ratios of $A_{260}/A_{280}$ for purified intact virus and core particles were 1.42 and 1.63, respectively. As expected, the core had a higher ratio than intact particles, presumably because the loss of the outer capsid had increased the proportion of nucleic acid.

Viral proteins

Protein from intact RGDV particles was resolved by polyacrylamide gel electrophoresis into two major [120 × 103 and 45 × 103 (K)], three intermediate (165K and 143K, and 56K) and two minor (183K and 150K) protein components (Fig. 3a, Fig. 4a). The relative amounts of each protein were 26:59:5:5:4:1:<1 for the 120K, 45K, 165K, 143K, 56K, 183K, and 150K proteins, respectively. Although the 183K and 56K proteins each sometimes appeared to be two close bands, we have considered them as being single proteins in this report. When the core particles were subjected to electrophoresis, the relative amounts of the 183K, 165K, 120K and 56K proteins were like those of intact particles (Fig. 3b), but the amounts of 150K, 143K and 45K proteins were much less than in intact particles. The 150K, 143K and 45K proteins did not sediment in CsCl gradients and were recovered from the tops of the gradients. The relative amounts of the 183K, 165K, 120K and 56K proteins in this fraction were negligible.
Proteins of RGDV

1.267
1.285
1.406
1.496

Density (g/ml)

pH 6.0 6.5 7.0 7.5 8.0 7.5

Fig. 1. Diagram of the light-scattering bands formed by equilibrium centrifugation of RGDV in CsCl gradients buffered with His-Mg at various pH values and photograph of a gradient at pH 7.5.

Fig 2. RGDV particles negatively stained with PTA. Left, intact particles; right, core particles. Bar markers represent 100 nm.

Immunoblotting

Immunoblotting showed that antiserum against intact RGDV reacted mainly with the 150K and 45K proteins (Fig. 4b), whereas the antiserum against RGDV core particles reacted mainly with the 183K and 120K proteins (Fig. 4c).

DISCUSSION

The RGDV capsid was found to be composed of two layers, the outer capsid and the inner capsid surrounding the RNA (Omura et al., 1982). The inner capsid plus RNA is described here as the core. Separation of the outer capsid layer from the inner core enabled us to locate the proteins in the respective components of the virus. The 183K, 165K, 120K and 56K proteins could always be detected in almost equal amounts in core particles and intact virus particles.
Fig. 3. Electrophoresis of RGDV protein in 10% polyacrylamide gel. (a) Protein from intact particles. (b) Protein from core particles. (c) Protein from the top fraction of gradients after CsCl equilibrium centrifugation.

Fig. 4. Immunoblotting of RGDV proteins. RGDV proteins were separated by electrophoresis in polyacrylamide gel and stained (a) or blotted onto nitrocellulose paper and treated with antiserum to intact particles (b) or antiserum to cores (c), and then stained with fluorescein-conjugated second antibody.

Table 1. Molecular weight and location of component proteins of RGDV

<table>
<thead>
<tr>
<th>Protein species</th>
<th>Presence in</th>
<th>Detection by antiserum to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus particle</td>
<td>Core</td>
</tr>
<tr>
<td>Mol. wt. (× 10^-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>183</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>165</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>150</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>143</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>56</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(Fig. 3b). On the other hand, the 150K, 143K and 45K proteins were found at the tops of the gradients after CsCl equilibrium centrifugation (Fig. 3c). These results suggest that 150K, 143K and 45K proteins were removed from the inner core particles.

The 150K and 45K proteins reacted strongly with antiserum to intact particles (Fig. 4b), whereas when antiserum against core particles was used, the 183K and 120K proteins reacted strongly (Fig. 4c). These results suggest that the outer capsid proteins are preferentially recognized as immunogens in the intact particles and that proteins of the core are not recognized unless the outer capsid is removed. Our results therefore suggest the following locations for the proteins: the 150K and 45K proteins are in the surface of the outer capsid, the 183K and 120K
Proteins of RGDV

Proteins are in the outer part of the core, the 165K and 56K proteins are inside the core, and the 143K protein is either on the inside of the outer capsid or within, but not on, the surface of the core (Table 1).

Two major protein bands have been observed in particles of three viruses that belong to the Phytoreovirus subgroup, i.e. 120K and 45K (Fig. 3a, 4a) in RGDV, 131K and 46K + 45K in RDV (Nakata et al., 1978) and 118K and 36K + 35K in WTV (Reddy & Macleod, 1976). It is interesting that the larger proteins are located in the cores and the smaller proteins are found in the capsid (Table 1; Nakata et al., 1978; Reddy & Macleod, 1976). The 45K protein of RGDV did not separate into two bands in our experiment, but the corresponding bands of RDV and WTV separated into two (46K and 45K in RDV, and 36K and 35K in WTV).

Treatment of particles with organic solvents removes the outer capsids of Fijivirus subgroup viruses but not those of viruses in the Phytoreovirus subgroup (Milne & Lovisolo, 1977; Omura et al., 1982). As was shown by Reddy & Macleod (1976) for WTV and by Nakata et al. (1978) for RDV, the outer capsid of RGDV was removed by CsCl treatment at an appropriate pH. These results suggest that the binding forces between the outer capsid proteins and the core proteins of virus particles in the Phytoreovirus subgroup and the Fijivirus subgroup are different. It is possible that studies of the removal of the outer capsid proteins by a combination of CsCl treatment and variations in the pH of the buffer may assist the study of the complex structure of these virus particles.

This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency of Japan.

REFERENCES


(Received 26 September 1984)