The Spike Protein of Potato Yellow Dwarf Virus and Its Functional Role in the Infection of Insect Vector Cells

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SUMMARY
Treatment of particles of potato yellow dwarf virus, serotype SYDV, with non-ionic detergents solubilized two of the five structural proteins, G and M1. Since only these proteins were also recovered in lipid vesicles prepared either by a detergent dialysis technique or after organic extraction of purified virus using phosphatidylcholine in n-hexane, it is suggested that M2 protein is not membrane-associated but rather belongs to the core. The SYDV spike protein G was purified by isoelectric focusing during which it appeared to be heterogeneous in charge with most of the protein banding at pH 4.8. The same heterogeneity was observed after two-dimensional protein gel electrophoresis, where SYDV G protein was found largely at pH 4.8, whereas that of the other PYDV serotype, CYDV, was detected largely at pH 4-3. Antibodies raised against purified SYDV G protein reacted specifically with the G protein. When they were present during the inoculation step, G-specific antibodies were able to neutralize the infectivity of SYDV for insect vector cell monolayers, which suggests that G protein has a functional role in the inoculation process. Isolated SYDV G protein or SYDV envelope vesicles containing G protein inhibited the infection of vector cells by SYDV; however, only with SYDV envelope vesicles was the inhibition concentration-dependent. This indicated competition between virions and the vesicles during the inoculation process, possibly for recognition or attachment sites.

INTRODUCTION
Established cell lines as well as primary cultures of insect vector cells can be infected in vitro with plant rhabdoviruses (Black, 1969; Peters & Black, 1970). Little, however, is known about the infection pathways of these viruses in their vector cells, which may be related to the mechanisms underlying virus vector specificity. By analogy with vertebrate rhabdoviruses, it could be assumed that the surface glycoprotein that forms the spikes of the virus particles is involved in this specificity (Wagner, 1975).

For potato yellow dwarf virus (PYDV) there is experimental evidence that these surface proteins play a functional role during the inoculation of insect vector cells since the two different serotypes of this virus have different pH optima for infection of the cells (Hsu & Black, 1973a), which correlates well with differences observed between their glycoproteins (Falk & Weathers, 1983; Adam & Hsu, 1984). In addition, it was observed that removal of the glycoproteins drastically reduced the infectivity of particles for the insect cells (Hsu et al., 1983) but not for the plant host when inoculated mechanically.

In order to examine a possible functional role of the PYDV glycoprotein during inoculation of insect vector cells, we have isolated the glycoprotein G from the serotype SYDV, raised antibodies against it, and have studied the effects of the isolated protein and the monospecific antibodies on the inoculation and infection of cells.

METHODS
Insect vector cell lines. The established insect cell lines, AC-20 from Agallia constricta and AS-2 from Aceratagallia sanguinolenta, were obtained from Dr H. T. Hsu, ATCC, as subcultures from the originally
established cell lines (Chiu & Black, 1967). The cells were maintained as described by Black (1979) using LB culture medium (Liu & Black, 1976). Cell monolayers were produced on glass coverslips (15 mm diam.) in CCSC culture vessels (Greiner, Nürringen, F.R.G.).

The inoculation of monolayers for bioassays and subsequent staining with fluorescein isothiocyanate-labelled antibodies were done as described by Hsu & Black (1973 a). As inoculation buffer, we used 0·1 M-histidine buffer containing 0·01 M-MgCl₂. The pH was adjusted to 5·9 by titration of histidine—HCl with histidine solution. For immunofluorescence microscopy we used an inverted microscope with incident u.v. light (Zeiss ICM 405), equipped with an ocular net-micrometer covering an area of 0·13 mm² at a magnification of 312·5 which was used for cell counting.

**Viruses.** The SYDV and CYDV serotypes of PYDV were propagated and purified as described by Adam & Hsu (1984). They were originally obtained from Dr H. T. Hsu.

**Isolation of the SYDV glycoprotein.** The SYDV glycoprotein, termed G protein, was isolated from purified virus as described by Dietzschold et al. (1978) with slight modifications. Virus in VP (0·1 M-m-glycine, 0·01 M-MgCl₂, pH 7·0) was mixed with an equal volume of 3% (v/v) Triton X-100, 7 mM-dithiothreitol (DTT). After 30 min incubation at 4 °C, the mixture was centrifuged to remove the liberated cores (Beckman SW56 rotor at 40000 r.p.m. for 1 h at 4 °C). The resulting supernatant fluid which contained the solubilized envelope proteins was dialysed extensively against 0·1 M-m-glycine, 0·1% Triton X-100 at 4 °C. The dialysed proteins were subjected to isoelectric focusing in a sucrose gradient column (L.K.B.), containing 0·1% Triton X-100. The pH gradient was generated with 2% Servalyte (pH 4 to 9) in 72 h with 400 V constant at 4 °C. Focused gradients were fractionated from the bottom. Fractions were monitored for absorbance at 280 nm and analysed for their protein content by SDS–polyacrylamide gel electrophoresis of samples precipitated from 10% TCA and washed twice with acetone. The main G protein-containing fractions were pooled, dialysed against 0·1 M-m-glycine, 0·1% (v/v) Triton X-100 and stored at 4 °C.

**Antibodies.** To produce antibodies, rabbits were injected subcutaneously first with G protein emulsified in complete Freund’s adjuvant. At 14 day intervals booster injections were administered using protein in incomplete adjuvant. For each injection 2 mg purified G protein was used. Sera were tested for G-specific antibodies by double diffusion.

For the determination of the G protein content in either virus or glycoprotein preparations, a double antibody ELISA test (Clark & Adams, 1977) was used with antibodies against purified SYDV glycoprotein G. The test was calibrated with a SYDV G protein preparation for which the concentration had been determined gravimetrically (Hsu et al., 1983). ELISA tests were performed in Immulon microtitre plates (Greiner) using alkaline phosphatase-conjugated second antibody.

**Electrophoretic methods.** One-dimensional electrophoresis of SDS-denatured proteins was as described by Laemmli (1970) in slab gels of 10% (w/v) acrylamide containing 2.8% (w/w) bisacrylamide.

Two-dimensional (2D) separation was performed essentially as described by O’Farrell (1975). A mixture of 2% Servalyte 3–10 and 2% Servalyte 5–8 was used to generate the pH gradient. Samples were prepared from 50 µl purified phosphorylated virus which was dissolved in 20 µl 2D sample buffer (O’Farrell, 1975) without heating. Separation in the first dimension was for 7400 Vh, which was sufficient for marker proteins (Serva, PTM 9) to focus. The second dimension electrophoresis was in 10% gels, as described above. Proteins were stained with either Coomassie Brilliant Blue R250 (Weber & Osborn, 1969) or silver nitrate (Wray et al., 1981).

Electrophoretic blotting from either one- or two-dimensional gels and subsequent treatment with antibodies was as described by Towbin et al. (1979). Horseradish peroxidase-labelled Protein A (Bio-Rad) was used as a probe and bound enzyme was detected using the HRP colour development reagent from Bio-Rad, containing 4-chloro-1-naphthol, as described by the supplier.

**Preparation of virus envelope vesicles.** Vesicles from SYDV envelope constituents were prepared from purified virus by the detergent dialysis method described for vesicular stomatitis virus (VSV) by Miller et al. (1980). Purified virus in VP, equivalent to 10 mg G protein, was diluted with the same buffer to 2 ml and mixed with 2 ml 0·12 M-octyl-β-D-glucoside (OG) in VP. After 1 h on ice with occasional shaking, the mixture was centrifuged to remove insoluble material (Beckman SW56 rotor, 40000 r.p.m. at 4 °C for 1 h). The supernatant fluid was dialysed at 4 °C for 36 to 48 h against sterile inoculation buffer until the solution became turbid, indicating the formation of vesicles. These were sedimented by centrifugation (Beckman SW56 rotor, 35 000 r.p.m. at 9 °C for 30 min), washed once with sterile inoculation buffer and, after a second centrifugation, re-dissolved in inoculation buffer and stored at 4 °C. The protein content of vesicle preparations was analysed by SDS gel electrophoresis and the amount of G protein was determined by ELISA.

**Organic extraction of SYDV proteins.** Organic extraction of viral proteins was as described by Montal et al. (1978). Two-hundred µl purified SYDV, containing 1 mg G protein, was pelleted (Beckman Ti60 rotor 30000 r.p.m. at 4 °C for 1 h) and the pellet was re-dissolved in 30 µl VP. After the addition of 1 ml n-hexane, containing 10 mg phosphatidylcholine, the mixture was sonicated for 4 min at 4 °C (Bandelin Sonorex, RK 100). Thereafter, 100 µl 1 M-MgCl₂ was added to the mixture which was vortexed for 2 min. The phases were separated by centrifugation in a swingout rotor at 2000 r.p.m. for 3 min at 4 °C, the upper organic phase was distributed in
PYDV spike protein

Fig. 1. SYDV G protein purification and analysis. Fractions of dissociated SYDV as well as G protein isolated by isoelectric focusing were analysed on 10% SDS-polyacrylamide gels. Proteins were either stained with Coomassie Brilliant Blue (a to e), or by the periodic acid Schiff's reagent procedure (f). The positions of the five structural proteins of SYDV are indicated by letters on the left and right. Due to separation on separate gels the positions differ between (a, b) and (c to d). (a) Proteins solubilized with 1.5% Triton X-100 and not pelleted by ultracentrifugation. This material served for further purification of G protein by isoelectric focusing. (b) Proteins sedimented by ultracentrifugation after Triton X-100 dissociation. (c) SYDV protein pattern before treatment with Triton X-100. (d, e) Material from the main G protein band after isoelectric focusing. (f) Same material as in (e), but stained for carbohydrates.

150 µl portions into test tubes, and the hexane evaporated with a gentle stream of nitrogen. After addition of 150 µl VP per test tube and subsequent sonication for 1 min as above, the resulting lipid vesicles were washed once with VP and stored in VP at 4 °C.

Statistical methods. Experimental data from bioassays were analysed either with the H-test of Kruskal & Wallis or, in cases where the alternative hypothesis could be specified as a trend hypothesis, with the related S-test of Jonckheere. Mathematical formulae and tabulated values were taken from Lienert (1973). Linear regression analyses were calculated according to the method of least squares (Documenta Geigy, 1969).

RESULTS

Isolation and characterization of the SYDV glycoprotein

Treatment of SYDV particles with 1.5% (v/v) Triton X-100 in the presence of 50 mm-glycine, 5 mm-MgCl₂ and 3.5 mm-DTT, pH 7.0, resulted in almost complete disintegration of the viral envelope. After the core particles had been removed by centrifugation, only two of the five viral proteins, G and M₁, remained in the supernatant fraction (Fig. 1a), whereas the pellet contained the other proteins together with some unsolubilized envelope proteins (Fig. 1b). After
isolectric focusing the bulk of the G protein was detected in fractions corresponding to an
isoelectric point of 4.8 in the pH gradient, although fractions in more basic regions also
contained G protein but only in minor amounts. Because the pH gradients were linear, we
assumed that equilibrium had been reached in the column and that proteins had reached their
respective isoelectric points. The G protein of the CYDV serotype could be isolated by the same
procedure. Like the SYDV G protein it was heterogeneous in charge but differed in the
isoelectric point of the main band, which was detected at pH 4.5.

Two-dimensional electrophoresis revealed that G protein species with differing isoelectric
points, ranging from 7.0 to 4.8, existed also in purified virus preparations of both serotypes. The
main SYDV G protein spot was detected at the same pH as in the preparative focusing, whereas
that of CYDV focused at a more acidic value of 4.3 (Fig. 2), substantiating the difference
observed for the isolated spike proteins. That the heterogeneity was not an artefact of the
electrophoretic method could be deduced from the spot pattern of marker proteins (Serva, PTM
9) which were used in sister gels.

Electrophoresis of purified SYDV G protein in SDS-polyacrylamide gels revealed that it co-
migrated with G protein from purified virus (Fig. 1 c, d) and staining with the periodic acid–
Schiff's reagent showed that it had retained a carbohydrate moiety (Fig. 1 e, f). No other
proteins were detected in the purified G protein preparations, even when the sensitive silver
staining procedure was employed.

**Antibodies against the SYDV glycoprotein**

Antibodies obtained from sera of rabbits immunized with the isolated G protein after the
second booster injection, had a titre of 1:64 against the isolated G protein as determined in
double diffusion tests. These antibodies did not react with extracts of healthy plants in double
diffusion or ELISA tests. Reactions with the G protein from the related PYDV strain CYDV
were not observed in either double diffusion tests or when Western blots of CYDV proteins were
Fig. 3. Western blot analysis of antibodies against the SYDV G protein. Proteins from purified SYDV (a, b) or CYDV (c) were separated on 10% SDS protein gels, blotted electrophoretically onto nitrocellulose and treated with either SYDV antibodies (a) or antibodies against the SYDV G protein (b, c). Letters on the left indicate the four structural proteins G, N, M₁ and M₂. The L protein could not be identified unequivocally.

Fig. 4. Protein pattern of SYDV envelope vesicles and lipid vesicles. Proteins were separated on 10% SDS protein gels and either stained with Coomassie Brilliant Blue (a to c) or with silver nitrate (d, e). The letters on the left indicate the position of the structural proteins. (a) Purified SYDV particles. (b) Supernatant fraction after ultracentrifugation of OG-treated SYDV particles. (c) Envelope vesicles formed during dialysis of (b) against inoculation buffer. (d) Buffer control. (e) Pattern of proteins from lipid vesicles made from organic extracts of purified SYDV as described in the text. The asterisk marks a band pattern present also in the buffer control (d), which presumably originates from the sample buffer.

treated with the antibodies (Fig. 3). However, in ELISA the CYDV G protein reacted with the SYDV antibodies although with 16-fold lower readings.

The additional bands above and below the G protein position in Fig. 3(b) were probably not due to non-specific reactions with other viral proteins, since G protein readily forms dimers
responsible for the upper band (Hsu et al., 1983) and degradation due to proteases would form fragments of higher mobility. We have been able to show that both bands contain carbohydrate and therefore probably are related to the only glycoprotein of the virus.

ELISA tests were also used to quantify G protein in virus preparations, G protein preparations and SYDV envelope vesicles. The test response was linear in the range from 5 to 500 µg/ml G protein, when plotted on a semi-log scale.

**Preparation of virus envelope vesicles**

To prepare virus envelope vesicles the detergent OG was used to dissolve the viral envelope because it could be removed by dialysis. Preliminary experiments had shown that 0.06 M-OG in VP was sufficient to dissolve the SYDV envelope. After centrifugation, the supernatant fraction of OG-disrupted virus contained the two viral proteins G and M₁ (Fig. 4b) together with the envelope lipids. When OG was removed by dialysis, the solution became turbid and material could be sedimented by ultracentrifugation, showing that vesicles had formed when the critical detergent concentration was reached during dialysis. As shown in Fig. 4(c) the generated vesicles contained the two proteins G and M₁ in almost the same proportion as in the virus (Fig. 4a). We have called such particles virus envelope vesicles because they consisted only of constituents of the viral envelope, and banded in Ficoll gradients at positions expected for lipid vesicles.

Lipid vesicles incorporating the envelope proteins of the virus particle were also obtained when purified virus was extracted with hexane containing phosphatidylcholine. The residue that remained after extraction and evaporation of the organic phase could be used to prepare lipid vesicles which contained the two viral proteins G and M₁ as determined by SDS-polyacrylamide gel electrophoresis (Fig. 4e). The G protein in the vesicle preparation reacted with G-specific antibodies in ELISA tests, indicating that the G protein had remained serologically active.

**Injectivity neutralization tests**

Neutralization with antibodies

The neutralizing ability of the monospecific antibodies against the SYDV G protein was tested by addition of purified IgGs at different concentrations to either purified SYDV or CYDV just prior to inoculation of the cell monolayers. The virus concentration in the inoculum was adjusted so as to result in 1 to 2% cells becoming infected when applied without IgGs. For control purposes inocula either without IgG or with preimmune serum at a dilution of 1:500 were used. As shown in Fig. 5 preimmune serum had no influence on the number of cells infected, whereas the addition of G-specific antibodies reduced the number of cells infected. The percentage inhibition, based on the preimmune serum control, increased linearly with increasing IgG concentration when plotted on a semi-log scale (Fig. 5). The increase in inhibition was highly significant at the 1% level when tested by the Σ-test, assuming that the inhibition increases with increasing antibody concentration. Infection by either the SYDV or the CYDV strain of PYDV was inhibited by SYDV G-specific antibodies, although the inhibition of infection by CYDV was about tenfold less sensitive than that of SYDV (Fig. 5). These results confirmed the cross-reactions observed in the ELISA tests but also revealed significant differences between the two virus strains.

**Inhibition of infection by G protein and virus envelope vesicles**

To determine whether the isolated G protein or envelope vesicles containing G protein could compete for the infection of cells with SYDV, we pretreated the cells for 30 min with either G protein or envelope vesicles in inoculation buffer, washed the cells with the inoculation buffer and then inoculated them for 45 min with virus particles. Virus inoculum was diluted to result in 1 to 2% infected cells. The amounts of G protein in the inocula and in the vesicle preparations were determined by ELISA prior to the experiments. Based on the amount of G protein in the inoculum, the amount of G in the pretreatment step was varied between 0.1 and 10 times that in
Fig. 5. Neutralization of infectivity by antibodies to SYDV G protein. Monolayers of AS-2 cells were inoculated with SYDV (■) or CYDV (○) in the presence of different concentrations of SYDV G antibodies. The percentage inhibition was based on the number of infected cells obtained after inoculation in the presence of preimmune serum. Each point represents the mean of three monolayers. The curves were fitted by linear regression analysis with r = 0.987.

Table 1. Effect of pretreatment with isolated G protein on SYDV infection*

<table>
<thead>
<tr>
<th>Ratio Gp/Gt</th>
<th>Infected cells (%)†</th>
<th>Inhibition (%)‡</th>
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<tr>
<td>0.0 (control)</td>
<td>2.6</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1</td>
<td>1.14</td>
<td>56</td>
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<tr>
<td>0.5</td>
<td>1.10</td>
<td>58</td>
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<tr>
<td>1.0</td>
<td>0.95</td>
<td>63</td>
</tr>
<tr>
<td>5.0</td>
<td>1.18</td>
<td>55</td>
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<tr>
<td>10.0</td>
<td>0.92</td>
<td>65</td>
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* AS-2 monolayers were pretreated either with inoculation buffer or isolated SYDV G protein for 30 min at 28 °C before they were inoculated with SYDV inoculum containing 3.3 μg/ml G protein (Gt). The amount of G protein in the pretreatment step was varied between 0.3 and 33 μg/ml (Gp) resulting in ratios Gp/Gt from 0.1 to 10. Using the statistical H-test for comparison of the different treatments an H of 8.68 was calculated; at the 5% level the differences are not significant.
† The values are the means from 20 microscopic fields counted on two monolayers/treatment.
‡ The percentage inhibition is based on the control treatment.

the inoculum. The following controls were included: (i) inoculation only with G protein or vesicles and (ii) pretreatment with the dialysis solutions of G protein or vesicles followed by inoculation.

No infected cells were observed when they were inoculated only with vesicles or G protein. Pretreatment with the dialysis solutions had no inhibitory effect. Pretreatment with isolated G protein caused a reduction in the number of cells infected; however, there was no correlation between inhibition and the concentration of G protein during the pretreatment (Table 1). Moreover, the differences between inhibition at different G protein concentrations were not significant when tested with the H-test. This was observed in two independent experiments with two different G protein preparations, but the level of inhibition differed in the two experiments. When vesicles were used instead of G protein, a decrease in the number of cells infected was observed with increasing amounts of envelope vesicles in the pretreatment step (Fig. 6). The S-test substantiated this decrease because it allowed acceptance of the hypothesis that an increase in inhibition was correlated with the increase of G protein-containing vesicles in the pretreatment step. A 50% reduction of infection was obtained when the pretreatment was carried out with the same amount of G protein as applied with the inoculum.

To determine which of the envelope vesicle constituents was responsible for the observed inhibition we used antibodies against the G and M protein as well as preimmune antibodies which were added during the pretreatment step. The pretreatment was performed with 10 times
Fig. 6. Inhibition of SYDV infection by SYDV envelope vesicles in two separate experiments. Monolayers of AS-2 cells were inoculated with SYDV. Prior to application of inoculum the cells were pretreated with SYDV envelope vesicles. The amount of G protein in the pretreatment step (Gp) was varied between one-tenth and 10 times of that of the inoculum (G0). The percentage infectivity was calculated on the basis of treatments with inoculation buffer. Each point represents the mean from two monolayers. The curve was fitted by a linear regression analysis with \( r = 0.96 \).

the G protein concentration of the inoculum and antibodies were present at 100 \( \mu g/ml \). The envelope vesicles reduced the number of cells infected to 50\% of the control. Addition of G antibodies raised this number to 75\% whereas no effect was observed with M1 or preimmune antibodies with which 40\% and 50\% of the cells were infected. This indicated that the observed inhibition is probably due to the presence of the G protein and not to the M1 protein.

DISCUSSION

As with other enveloped viruses of both plants and vertebrates, treatment of PYDV particles with non-ionic detergents caused their fractionation into soluble proteins belonging to the envelope and a nucleoprotein complex. In our experiments two PYDV proteins, G and M1, always became solubilized together whichever detergent was used. Almost the same result was obtained by Falk & Tsai (1983). When they treated PYDV with NP40 mainly G and M1 and only minor amounts of M2 protein became solubilized. A further indication that G and M1 of PYDV are the only membrane-associated proteins was that only these two proteins were extracted by a method using organic solvents which is known to liberate membrane proteins from bacteria (Montal et al., 1978) or in a modified version from influenza virus (Gregoriades, 1980). These results are in accordance with the suggestion of Zaides et al. (1979) that the hitherto accepted classification of the rabies virus proteins (Wagner et al., 1972) according to which M1 and M2 are both membrane-associated proteins should be re-evaluated. Although it might well be that we have not used conditions stringent enough to obtain complete solubilization of the PYDV membrane proteins, it is tempting to follow the suggestion of Zaides et al. (1979) that one of the so-called membrane proteins in fact belongs to the core. This suggestion, and our results with SYDV dissociation, are further corroborated by results of Peters et al. (1978) who reported for plant viruses of the lyssavirus subgroup that the M protein present in abundance (either M1 or M2) always solubilized together with the G protein.

The isolated G proteins of both PYDV strains, SYDV and CYDV, revealed a remarkable charge heterogeneity which was also found when virus was dissociated and subjected directly to 2D gel electrophoresis. Since both methods revealed the CYDV G protein to be more acidic than the SYDV G protein, a difference in charge can be assumed. Correction of the apparent isoelectric points obtained from the 2D electrophoresis for the influence of urea present in the focusing step by a factor of 0.5 (Gelsema et al., 1979) led to values of 4.3 and 3.8 for SYDV and CYDV G protein, respectively. The differences between the values obtained for the isolated proteins without urea and the corrected values from 2D electrophoresis might be due to conformational changes during exposure to urea as suggested by Gelsema et al. (1979). The charge heterogeneity of the PYDV G proteins resembles those reported for the glycoprotein of
PYDV spike protein

VSV (Hsu & Kingsbury, 1982) and other enveloped viruses (Raghow et al., 1978), but in contrast to that of VSV, the SYDV G protein is rather acidic as predicted from its amino acid composition (Knudson & MacLeod, 1972).

Antibodies prepared against the SYDV G protein were highly specific for this protein and did not react with other SYDV proteins nor with the related CYDV strain G protein when tested on Western blots of separated proteins. In contrast to such SDS-denatured proteins, ELISA tests revealed a cross-reaction between G proteins of SYDV and CYDV which agrees with results from Falk & Weathers (1983) obtained by the same technique.

For bioassays, we have chosen the rather low level of 1 to 2% infected cells. This was done mainly because of two reasons: first cell counting is easier and therefore less prone to counting errors when the number of infected cells per counted field is low, which means in the above range five to ten cells; second, experiments by Hsu (1978) have shown that high multiplicities of infection led to cytopathic effects that are due not only to the infection but also to virus components of the inoculum. To avoid such interference the low infection level was used. Nevertheless, counting of ten microscopic fields per coverslip with two coverslips per treatment was sufficient to discriminate between the different treatments as can be judged from the statistical treatment of the data. This is mainly due to the low levels of variation that can be obtained with the test system (Hsu & Black, 1973b). We did not reach such low coefficients of variation but typically we obtained a mean of 500 cells per counted area (s.d. 30) and five infected cells (s.d. 1-5).

Neutralization of SYDV infectivity by virus-specific antibodies has already been reported by Liu & Black (1978); however, since they used antibodies against whole virus it was not possible to attribute the neutralizing activity to a particular viral protein. In our experiments the observed neutralization can clearly be attributed to the G protein which is in good accordance with results reported for other rhabdoviruses like VSV (Kelley et al., 1972) and rabies virus (Cox et al., 1977) where G protein was found to be essential for infectivity. Because in our experiments the G-specific antibodies were present only during the inoculation step, it can be assumed that an early step of the infection process is affected by the antibodies, possibly recognition of, or attachment to, the host cells. These are the generally accepted functional roles for the G protein of VSV (Wagner, 1975).

Further support for the functional identity between the SYDV spike protein and those of the vertebrate rhabdoviruses came from our experiments where cells were treated prior to infection with either SYDV G protein or SYDV envelope vesicles. Pretreatment with G protein inhibited independently of the amount of G protein, offering two possible interpretations. Either the protein concentrations were still too high or the G protein affects cellular processes rather than the virus-cell interaction. We would rather favour the latter, because Thimmig et al. (1980) have shown for VSV that there is no competition in binding between G protein and VSV particles. An explanation for the inhibition we observed could then be the inhibition of cellular nucleic acid synthesis by isolated G protein as described by McSharry & Choppin (1978) for VSV. In contrast to the observation with G protein, envelope vesicles inhibited infection in a concentration-dependent manner as has been reported for VSV by Miller et al. (1980). Although the SYDV envelope vesicles used in our studies contained two proteins, G and M1, whereas the VSV envelope vesicles prepared by Miller et al. (1980) contained only G, the partial reversal of the inhibitory effect of SYDV vesicles by G-specific antibodies but not by preimmune or M1 antibodies indicated that the observed inhibition in our experiments is also due to the G protein. We cannot exclude additional interference by the lipids of the vesicles because the reversal by the G antibodies was not complete. The difference between the effect of G protein and envelope vesicles might be due to the different presentation of the G protein to the cell. Because of similarities in the arrangement of the G proteins in the virus particles and envelope vesicles, competition could be expected.

Whether competition between SYDV envelope vesicles and SYDV particles occurred on the cell surface or at internal membranes remains to be determined. However, the sensitivity of SYDV infection to lysosomotropic agents such as chloroquine or NH4Cl, as reported in the accompanying paper (Adam & Gaedigk, 1986), indicates that SYDV enters the vector cells by a
pathway similar to that suggested for VSV (Miller & Lenard, 1980; Matlin et al., 1982), rabies virus (Superti et al., 1984) and influenza B virus (Shibata et al., 1983). It is therefore possible that competition occurs inside the cells as reported for VSV by Miller et al. (1980).

The possibility that the observed pathway of PYDV infection of vector cells also regulates virus vector specificity is the subject of further studies including both serotypes of this virus which are transmitted differentially.

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