Early Events in the Infection of Tobacco with Alfalfa Mosaic Virus

(Accepted 20 July 1978)

SUMMARY

At 23 °C, infections with alfalfa mosaic virus can be initiated with the three genomic RNA species in combination either with coat protein or with the messenger for coat protein (RNA 4). At 30 °C the combination of the three genomic RNA species with coat protein is still infectious to tobacco, whereas the infectivity of the combination with RNA 4 is almost nil. The combination of the four RNA species is infectious to bean at both temperatures. Tobacco leaf discs inoculated with the four AMV RNA species were incubated for short periods at 30 °C followed by 4 days at 23 °C. Infectivity could only be recovered when the shift-down occurred within 10 min after inoculation. Longer exposure to 30 °C probably leads to the degradation of one or more of the AMV RNA species. Translation of RNA 4 into coat protein at 23 °C in the inoculated cells presumably will render the RNA infection resistant to 30 °C. To measure the minimum time required for the penetration and translation of RNA 4, tobacco leaf discs inoculated with the four AMV RNA species were incubated for different periods at 23 °C and then for 4 days at 30 °C. From the results it was estimated that 15 to 30 min is sufficient for the penetration and translation of RNA 4.

Alfalfa mosaic virus (AMV) is a plant virus with a coat protein-dependent tripartite genome. At ambient temperatures infections of several hosts can be initiated with a combination of the three genomic RNA species and coat protein (supplied either as nucleoprotein particle or as soluble protein), or with a combination of the three genomic RNA species and RNA 4 (Bol et al. 1971). The finding that RNA 4 contains the message for the coat protein (Van Ravenswaay Claasen et al. 1967; Mohier et al. 1975; Thang et al. 1975; Van Vloten-Doting et al. 1975; Rutgers, 1977) supports the postulate that upon inoculation with the four AMV RNA species, RNA 4 is translated into coat protein and that it is this coat protein which activates the genome (Bol et al. 1971).

Recently I have observed that the combination of the three genomic RNA species and RNA 4 failed to infect tobacco (Nicotiana tabacum L. cv. Samsun NN) at 30 °C, while the same mixture was infectious to tobacco at 23 °C and to bean (Phaseolus vulgaris L. cv. Berna) at both 23 and 30 °C. Moreover a combination of the three genomic RNA species and coat protein as well as a combination of the three nucleoproteins was infectious to both tobacco and bean at both temperatures (Table 1). Similar observations were made for tobacco streak virus, another virus with a coat protein-dependent tripartite genome (Van Vloten-Doting, 1975).

Two sets of experiments were performed to investigate this phenomenon. For all experiments, material from AMV strain 425 was used. Virus nucleoprotein, RNA and coat protein were isolated as described before (Van Vloten-Doting & Jaspars, 1972; Bol & Van Vloten-Doting, 1973). Tobacco leaves, dusted with carborundum, were inoculated with a mixture of the four AMV RNA species at a concentration of 1 mg/ml. This concentration will
Table I. Effect of temperature on the infectivity of AMV nucleoproteins and AMV RNA species on tobacco and bean

<table>
<thead>
<tr>
<th>Host</th>
<th>Temp. (°C)</th>
<th>Inoculum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco*</td>
<td>23</td>
<td>RNA</td>
<td>178</td>
<td>127</td>
</tr>
<tr>
<td>Tobacco*</td>
<td>30</td>
<td>Nucleoprotein</td>
<td>1</td>
<td>104</td>
</tr>
<tr>
<td>Bean†</td>
<td>23</td>
<td>RNA</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>Bean†</td>
<td>30</td>
<td>Nucleoprotein</td>
<td>59</td>
<td>58</td>
</tr>
</tbody>
</table>

* After 4 days, virus production was measured by local lesion assay on bean. Numbers are mean values for eight half-leaves.
† Lesions were counted after 2 days. Numbers are mean values for six half-leaves.

induce a nearly confluent pattern of symptoms on tobacco at 23 °C. Directly after inoculation, leaf discs (1 cm diam.) were punched out. The discs were divided randomly between 50 ml Erlemeyer flasks (10 disc/flask) containing 20 ml 0.01 M-NaH₂PO₄, pH 7.0, pre-incubated at 30 °C. The samples were incubated for variable periods at 30 °C followed by 4 days of incubation at 23 °C under continuous illumination of 3000 lux (TL tubes colour no. 33). At the end of the incubation each sample of ten discs was ground in a mortar with 2 ml of PEN buffer (0.01 M-NaH₂PO₄, 1 mM-ethylenediaminetetraacetic acid, 1 mM-NaNO₃, pH 7.0). The homogenate was passed through aseptic gauze and diluted 3-, 9- and 27-fold with PEN buffer. Each dilution was compared to the corresponding dilution of the sample incubated continuously at 23 °C, by infectivity assay on bean. Only lesion numbers below 200 per half leaf were taken into account. The mean value of each dilution was expressed as percentage of the mean value of the same dilution of the control. From Fig. 1 (©-©-©) it can be seen that, in tobacco leaves at 30 °C, the infectivity of the AMV RNA species is lost within 10 min. In the literature the estimates of the survival time of inoculated virus RNA under non-replicative conditions vary from half an hour to several hours. U.v.-irradiated tobacco mosaic virus RNA could be nearly completely photoreactivated at 20 °C when light was given directly after inoculation. Delay of the light treatment for half an hour resulted in less photoreactivation, while no photoreactivation was found when the interval between inoculation and photoreactivation was 1 h (Bawden & Kleczkowski, 1966). Bawden & Sinha (1961) found that neither red clover mottle virus (RCMV) nor its RNA were infectious to French bean at 32 °C. Shift-down experiments showed that inoculated virus could survive at 32 °C for at least 22 h, while the infectivity of inoculated RNA was lost within 5 h. Kassanis (1962) reported that inoculated satellite virus RNA is unstable and has to be rescued by inoculation with tobacco necrosis virus (TNV) within 1 h to be an effective inoculum. However, occasionally, inoculation of TNV 2 days after satellite virus (SV) RNA resulted in the production of SV. Apparently RNA can sometimes be protected from inactivation in vivo by cellular components. It is possible that the low level of infectivity found occasionally in tobacco inoculated with the four AMV RNA species and kept at 30 °C is due to the same phenomenon.

In the reverse experiment, leaf discs inoculated with the four AMV RNA species were incubated, first at 23 °C followed by a 4-day incubation at 30 °C. The amount of virus produced in each sample was measured as described above. Fig. 1 (■—■) shows that an incubation of 20 min at 23 °C will result in the formation of a small amount of virus, while discs incubated for 40 min at 23 °C followed by 4 days at 30 °C contained even more virus than control discs kept continuously at 23 °C. This experiment was repeated several times.
Fig. 1. Effect of the time of incubation at 30 °C (○—○) or 23 °C (■—■) on the amount of virus produced in tobacco leaf discs inoculated with AMV RNA and kept for an additional 4 days at 23 °C (○—○) or 30 °C (■—■). The amount of virus produced was measured by local lesion assay. Leaf discs kept continuously at 23 °C were used as controls.

Although there is some difference in the time of appearance of the infectivity (4 to 20 min), the shape of the curves was the same as the curve in Fig. 1. These experiments show that after the infection with the four AMV RNA species there is a critical step, which takes between 4 and 20 min, and that after this time the leaves inoculated with the four AMV RNA species will produce virus at 30 °C. As it was shown that addition of coat protein to the AMV RNA species allows them to infect tobacco plants at 30 °C (Table 1), it is plausible to assume that this critical step involves the translation of RNA 4 into coat protein.

From the observation that the RNA can survive for up to 10 min at 30 °C (Fig. 1, ○—○) we may conclude that RNA 4 is not sufficiently translated into coat protein during this period to render the infection resistant to incubation at 30 °C. Since 4 min at 23 °C can be sufficient to permit subsequent replication at 30 °C, either the translation at 23 °C is more rapid or, more likely, the translation of RNA 4 is completed during the first 10 min after the shift-up to 30 °C. If the 2 min required for inoculation are also taken into account, it may be concluded that translation of RNA 4 is likely to occur within 16 min after inoculation.

The translation rate for the α and the β chain of haemoglobin in intact reticulocytes has been estimated at several temperatures (Hunt et al. 1969). From these data it can be calculated that in reticulocytes at 23 °C the formation of a protein of the size of AMV coat protein would require about 3 to 5 min.

The lack of infectivity for tobacco of AMV RNA at 30 °C could be due to the fact that at that temperature the inoculated RNA species are so quickly degraded that by the time coat protein is made under the direction of RNA 4, (allowing the virus replication to start)
one, or all of the AMV RNA species have been degraded so far that all, or nearly all, infectivity is lost. Apparently, at 23 °C this balance is less unfavourable in tobacco, while in bean the genomic RNA species survive at both temperatures until coat protein is made.

The large amount of virus produced after a short period at 23 °C followed by incubation at 30 °C is very remarkable. This result is reproducible and cannot be due solely to the inhibition of the plant defence mechanism at 30 °C (De Leeuw, 1968; Wu et al. 1969) since leaves inoculated with AMV nucleoprotein and kept at 30 °C will produce about 60 to 80% of the amount of virus produced at 23 °C (Table 1). At present it is unknown whether this increase in virus production is due to an increased number of primarily infected cells or to an increased amount of virus produced per cell.

I am grateful to Lyda Neeleman, Peter van’t Sant, Aad Wesseling, John Bol and Gied Jaspars for their experimental or theoretical contributions. This work was sponsored in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

Department of Biochemistry,  
State University of Leiden,  
P.O. Box 9505, 2300 RA Leiden,  
The Netherlands

REFERENCES


(Received 7 June 1978)