Importance of Capsid Integrity for Interference Between Two Isolates of Barley Yellow Dwarf Virus in an Aphid

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ABSTRACT

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Recently we showed that fewer aphids (*Macrosiphum avenae*) transmitted the PAV isolate of barley yellow dwarf virus (BYDV) if they had first acquired the MAV isolate, than if they had previously fed on healthy oats or on oats infected with other BYDV isolates. MAV irradiated with ultraviolet light for 30 sec or more, no longer interfered with PAV transmission when the two viruses were injected simultaneously into aphids. Irradiation of MAV also decreased both MAV transmission by injected aphids and recognition by MAV-specific antibody; these results indicate alteration of MAV capsid conformation. No morphological differences between particles irradiated for 15–60 sec and those of nonirradiated controls were detected by electron microscopy. Some particles irradiated for 120 sec appeared to be swollen. Several minutes of irradiation were required for particle disruption. We suggest that the altered MAV particles do not attach to receptors in aphid salivary glands and thus are neither transmitted nor able to compete with PAV for common receptor sites that recognize both viruses.

Adsorption of specific virus isolates to plasmalemma-bound receptors, a prerequisite for virus penetration into cells of several vertebrate systems, is dependent upon receptor recognition of the virus capsid (3). Serologically similar isolates of both enteroviruses (2) and rhinoviruses (10) compete for attachment to cell receptors. Studies utilizing chemically altered virus particles have demonstrated the role of virus coat protein in the recognition phenomenon. Chemical treatments that alter coat protein structure prevent viral attachment to host cells (13); treatments that destroy infectivity by altering only viral RNA, did not prevent attachment of the inactivated particles (1). Ultraviolet (UV) irradiation of virus suspensions induces changes in capsid protein structure. In a study of poliovirus, Katagiri et al (7) report a 10% loss in antigenicity following UV-irradiation of poliovirus suspensions for 10 min, and a 50% loss after 25 min. Infectivity of the same virus suspensions, however, was totally destroyed by only 1–2 min of UV. Loss of infectivity preceded observed decreases in antigenicity.

Rapid loss of infectivity following UV-irradiation of plant viruses for short periods or at low energy levels is well known (9,12,14). Many reports of UV effects on plant viruses, however, are studies of tobacco mosaic virus (TMV) or isolated TMV-RNA (11,19). Little information is available on the effects of UV on other types of plant viruses.

Barley yellow dwarf virus (BYDV), type member of the luteovirus group, is an isometric RNA virus which is transmitted in a persistent-circulative manner by aphids. Five serologically distinct isolates, transmitted in a vector-specific manner by four species of aphids, have been identified (15). In a previous paper (4) we described transmission interference between two serologically similar isolates of BYDV (MAV and PAV) in their aphid vector, *Macrosiphum avenae* (F.) (= *Sitobion avenae* [F.]). We suggested that the interference may result from competition between MAV and PAV for receptors, located in the aphid salivary gland, that regulate movement of virus through the gland and out of the aphid. In this report we describe results of preliminary studies on the importance of MAV capsid structure for both aphid transmission and for its role in MAV-PAV interference.

MATERIALS AND METHODS

The source of aphids, the BYDV isolates, and the methods used in this study have been described (4). To test the effects of ultraviolet radiation (UV) on MAV, 1-ml samples of purified MAV (175 μg/ml in 0.1 M phosphate buffer, pH 7) were placed in plastic dishes and irradiated for various time periods at 4 C with a 15-W Sylvania mercury vapor lamp (G-15T8) placed 10 cm above the sample. Incident radiation at the sample was 1.9×10^4 ergs/sec/cm^2; measured with an ultraviolet intensity meter (Ultraviolet Products Inc., San Gabriel, CA) with peak sensitivity at 254 nm. The virus suspension in the dish (3–4 mm deep) was

agitated continuously during treatment. Virus used as controls was treated similarly, but was removed from a dish prior to UV exposure. Following UV treatment, samples of MAV were diluted in the buffer, and injected into aphids (*M. avenae*), which were given a 5-day inoculation test feeding singly on healthy 'Coast Black' oat seedlings (*Avena byzantina* Koch) to evaluate virus transmission. Similar samples were examined by electron microscopy for particle morphology, and by enzyme-linked immunosorbent assay (EIA) for changes in antigenicity (15). To determine the effect of irradiated MAV on PAV transmission by *M. avenae,* samples of MAV irradiated for various times were mixed with unirradiated suspensions of PAV and buffer to give a final concentration of 130 μg MAV per milliliter and 30 μg PAV per milliliter. The samples containing both MAV and PAV were injected into aphids, which were then given an inoculation test feeding on oats. Transmission of MAV and PAV by these single, injected aphids was then compared as previously described (4).

RESULTS

In a preliminary study, single aphids injected with untreated MAV, transmitted virus to all 40 oat plants on which they had fed. None of 40 plants fed on by aphids injected with MAV UV-irradiated for 2, 5, or 15 min became infected. Enzyme immunosorbent assays (EIA) of the irradiated and control virus preparations indicated decreased virus-antibody binding with increased exposure to UV. Absorbance by EIA reactants (at 405 nm with a 1-mm light path) decreased from 0.605 to 0.487 when samples of MAV irradiated for 0–5 min were tested. This decrease in absorbance was equivalent to an 80% decrease in virus concentration in unirradiated control samples. Electron microscopic (EM) examination of MAV particles following negative staining with 2% phosphotungstic acid or uranyl acetate, indicated progressive breakdown and eventual dissolution of virus particles with increasing exposure to UV. Virus particles in control samples were hexagonally shaped with sharply defined margins, indicating that stain did not penetrate into the MAV particles. The MAV sample irradiated for 2 min contained particles of two types: particles 26 nm in diameter similar to those of controls; and swollen particles, 28–30 nm in diameter, with a rounded appearance. The sample irradiated for 5 min contained mostly rounded, swollen particles penetrated by stain. The sample irradiated 15 min contained particles in various stages of disintegration and aggregations of broken particles into irregular amorphous masses. These results indicated that UV-irradiation of MAV for 2 min caused morphological alterations of the virus particles, and a reduction in antibody recognition. The irradiated MAV particles did not interfere with PAV transmission when both were injected simultaneously into aphids. When 40 aphids were injected with PAV alone, 23 transmitted virus; in parallel tests of PAV mixed with irradiated MAV (2 min), 24 of 40 aphids transmitted PAV. Only 5 of 40 aphids transmitted PAV when untreated MAV was mixed with PAV before injection.

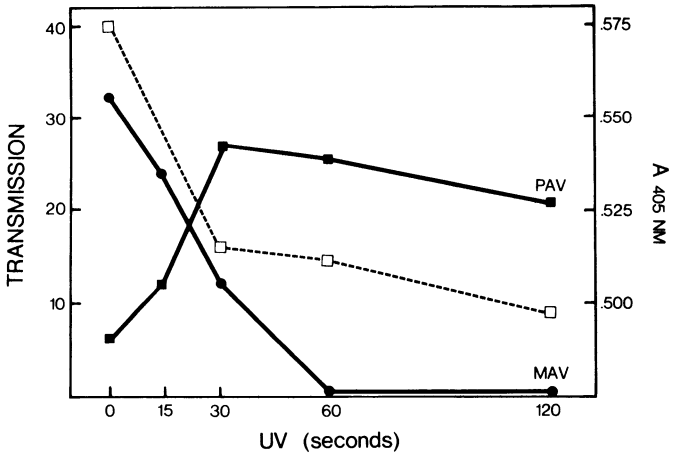

Fig. 1. Effect of ultraviolet irradiation (15–120 sec) on three properties of the MAV isolate of barley yellow dwarf virus: ●——● effect on transmission of MAV by injected *Macrosiphum avenae* (number of 40 that transmitted virus); ■——■ effect of irradiated MAV on PAV transmission when both viruses were injected simultaneously into 40 *M. avenae*; and □------□ effect of UV on the reaction of MAV in enzyme-linked immunosorbent assays measured by absorbance of reactants at 405 nm with a 1-mm light path. Absorbance of a control preparation of healthy oats was 0.01. The MAV preparation was irradiated at a concentration of 175 μg/ml in 0.1 M phosphate buffer (pH 7).

To study the effect of shorter irradiation times on MAV transmission and MAV interference of PAV transmission, samples of MAV were UV-irradiated for 0, 15, 30, 60, and 120 sec. Each sample was examined for particle morphology, for changes in antigenicity, and for transmission to oats by single aphids. Interference with PAV transmission by irradiated MAV was tested by mixing samples of treated MAV and unirradiated PAV, injecting the viruses simultaneously into aphids, and allowing the aphids to feed individually on oats, as previously described. All infected plants were tested to determine which ones were infected only with MAV (PAV interference), and which were infected with MAV and PAV (no interference).

Transmission of MAV again decreased with increasing UV-irradiation (Fig. 1). Particles of MAV irradiated for 15, 30, or 60 sec did not differ morphologically from unirradiated particles. The 26 nm particles in these samples showed no signs of swelling. A few particles irradiated for 120 sec were swollen and rounded. Quantitative techniques to distinguish such differences in particles among treatments were not attempted; differences were not obvious. Although gross morphological changes in MAV structure were not apparent, serological tests indicated a rapid decrease in recognition by MAV-specific antibody. These results indicated that changes in capsid conformation occur during short irradiation time (Fig. 1).

As reported previously (4), transmission of PAV is inhibited by high MAV concentrations when both MAV and PAV are injected into aphids. This result is shown again by transmission of PAV in the 0-sec control sample (Fig. 1). As MAV transmission decreased, there was a corresponding increase in PAV transmission. Transmission of PAV from samples mixed with MAV irradiated for 30, 60, and 120 sec was equivalent to transmission from a control sample containing only PAV (19 of 40 aphids transmitted (PAV). Thus, MAV did not interfere with PAV transmission when MAV was UV-irradiated for 30 sec or more. This experiment was repeated with different colonies of aphids, and MAV concentrations of 110 μg/ml with PAV at 20 μg/ml. Results were almost identical to those shown in Fig. 1. With increased UV-irradiation, MAV reactions in EIA tests decreased, as did MAV transmission. Decreased MAV transmission was associated with an increase in PAV transmission when MAV and PAV were injected simultaneously into aphids.

DISCUSSION

Interference with PAV transmission by high concentrations of MAV is believed to result from competition between the two isolates for receptor sites on aphid salivary glands (4). Visualization of the transmissible MAV isolate, but not of a nontransmissible isolate, in accessory salivary glands of *M. avenae* gave added support to this idea (5). In this study, UV-irradiated MAV did not interfere with PAV transmission. Although the MAV particles irradiated for 15–60 sec were not visibly altered, results of serological tests suggested at least minor changes in capsid conformation. It is possible that cell receptors no longer recognize and adsorb the altered MAV particles; thus, altered MAV could not compete with PAV for receptor sites. The PAV isolate would then move unimpeded through the salivary gland and be transmitted. The idea that UV-altered MAV particles are not recognized by cell receptors is consistent with results of a previous study in which MAV was treated with various chemicals that alter proteins (16). Some treatments prevented transmission of MAV without altering the sedimentation characteristics of the virus in

sucrose gradients.

Ultraviolet radiation could affect the virus particles directly by effects of radiant energy on aromatic amino acids, peptide bonds, and especially cystine residues; or indirectly by production of free radicals and oxidizing agents in the aqueous buffer in which the virus was suspended (6,18). Disruption of only one or a few bonds might be sufficient to alter tertiary structure and destroy the capsid conformation needed for recognition of the virus by specific receptors or antibodies. Effects of UV on plant virus protein structure, resulting in loss of infectivity, antigenicity, and viral components have been described (8,11,19). Our results suggest similar effects on MAV. Since so little is known about MAV structure (17), more work is necessary to determine the mechanism of UV-inactivation.

These results support the concept of the importance of coat protein structure in determining the MAV-PAV interference in aphids, and are compatible with the idea of aphid membrane virus receptors that regulate the transmission of plant luteoviruses.

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