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Short Communication

Inhibitory effects of 9-(2,3-dihydroxypropyl) adenine and 3-(adenin-9-yl)-2-hydroxypropanoic acid 2-methylpropylester on potato virus X replication

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Summary

9-(2,3-Dihydroxypropyl)adenine (DHPA) and 3-(adenin-9-yl)-2-hydroxypropanoic acid 2-methylpropylester (AHPA-MP)) markedly inhibit the replication of potato virus X (PVX). The latter compound inhibits virus replication more effectively at the lower concentration range. In synchronized virus-infected leaf-disks, the time–response course of inhibition by both compounds is almost identical, and similar to that of cycloheximide.

9-(2,3-Dihydroxypropyl)adenine (DHPA); 3-(Adenin-9-yl)-2-hydroxypropanoic acid (AHPA); Potato virus X (PVX)

Introduction

Although effective antiphytoviral compounds could be of substantial practical importance, the major effort in the field of antivirals has been aimed so far at the development of drugs which control the replication of vertebrate viruses. Nevertheless, several promising antiphytoviral substances have recently been described (for review, see Schuster, 1983, 1988). Particular attention has been paid to the search for antiphytoviral agents related to antimetabolites of nucleic acids biosynthesis. Analogs of purine and pyrimidine bases, nucleosides and nucleotides, and

their precursors and/or catabolites have been pursued as antiphytovirals (Hecht and Diercks, 1978; Ralph and Wojcik, 1976; Schuster et al., 1987; Schuster and Byhan, 1980; Schuster, 1988). Though efficient plant virus inhibitors, e.g. 5-azadihydrouracil (2,4-dioxohexahydro-1,3,5-triazine, DHT), are often nearly or completely inactive against vertebrate viruses (Schuster et al., 1979; Schuster and Byhan, 1980), certain antivirals active against animal and human viruses do exhibit an activity against some plant viruses as well. For example, ribavirin has proved effective against a great number of plant viruses (for review, see Hansen, 1988). Also (*RS*)-9-(2,3-dihydroxypropyl)adenine (DHPA), an adenosine analogue in which sugar moiety is replaced by an hydroxylated aliphatic chain, inhibits multiplication of several DNA and RNA viruses (De Clercq et al., 1978; Kara et al., 1979; De Clercq et al., 1984) as well as to tobacco mosaic virus, cowpea chlorotic mottle virus (Dawson, 1984), potato virus X, eggplant mosaic virus and a potyvirus isolated from *Solanum palinacanthum* (De Fazio et al., 1987). This drug affects methylation processes via suppression of *S*-adenosyl-L-homocysteine hydrolase, *S*-adenosyl-L-homocysteinase (SAHase) (Votruba and Holý, 1980), the time course of its action on the replication cycle of vaccinia virus suggests that viral mRNA is most probably the viral component affected by the drug (Rada et al., 1980). In addition to DHPA, 3-(adenin-9-yl)-2-hydroxypropanoic acid (AHPA) is also a potent irreversible inactivator of SAHase (Holý et al., 1985). AHPA esters acting as prodrugs of the parent acid with enhanced cell membrane penetrability inhibit the replication of a variety of animal and human viruses at significantly lower doses than DHPA (De Clercq and Holý, 1985). From these esters, AHPA 2-methylpropylester (AHPA-MP) was selected for further studies because of its rather high selectivity index. It was examined for its activity against potato virus X in comparison with DHPA and, as the time-course of its antiviral effect was followed, some information was obtained as to the site of action of these drugs in the virus replicative cycle.

Materials and Methods

Chemicals

9-(*RS*)-(2,3-Dihydroxypropyl)adenine (DHPA) was prepared as described by Holý (1975). (*RS*)-3-(adenin-9-yl)-2-hydroxypropanoic acid 2-methylpropylester (AHPA-MP) was synthesized as described by De Clercq and Holý (1985).

Virus and virus host; inoculation procedure and application of substances

Potato virus X (PVX), ringspot strain H 19, and *Nicotiana tabacum* L. 'Samsun' were used in the experiment. Plant cultivation and virus inoculation were described previously (Schuster et al., 1987). Tissue disks (13-mm diameter) were punched out with a cork borer. Each sample contained 20 disks. Immediately after this procedure (Fig. 1) or after having floated on water for different times (Fig. 2), the disks were vacuum-infiltrated with the virus inhibitors – the controls with water – and then laid on top of inhibitor solutions (or water) in 10-cm diameter

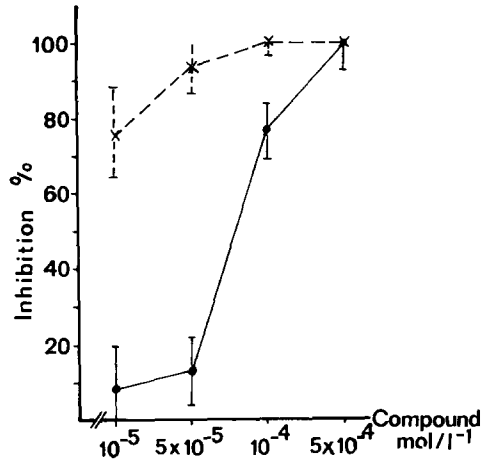

Fig. 1. Inhibition of PVX replication in disks of leaves of *Nicotiana tabacum* 'Samsun' by DHPA (•——•) and AHPA V-MP (x-----x).

Petri dishes. All experiments were repeated four times (80 disks were used for each treatment). The whole trial was repeated three to four times, so that, altogether, 240 to 320 leaf disks were used for one treatment.

Production of synchronous virus synthesis and estimation of the time-course of inhibition

Synchronous virus synthesis was achieved by a differential temperature inoculation (DTI) procedure originally described by Dawson and Schlegel (1973, 1976)

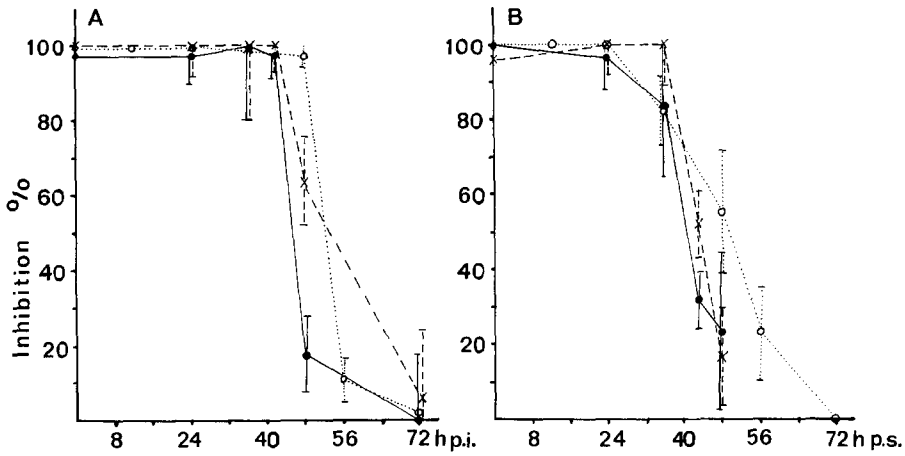

Fig. 2. Time-response curves of inhibition of PVX replication in disks of leaves of *Nicotiana tabacum* 'Samsun' by 5×10^{-4} mol·l^{-1} DHPA (•——•), 10^{-4} mol ·l^{-1} AHPA-MP (x-----x) and cycloheximide (5×10^{-4} mol·l^{-1}, ; o...o), without (A) or with (B) preincubation of the leaf disks for 4 days at 5°C; h.p.i. = hours after infection; h.p.s. = hours after the shift to permissive temperature.

and Dawson et al. (1975), and slightly modified by Schuster and Arenhövel (1984). Immediately after inoculation the leaves were exposed to low temperatures (5 ± 2°C), which arrest virus multiplication at a very early stage. After five days the leaves were shifted to permissive temperatures. Tissue disks were punched out and sampled as described above. The test substances were vacuum-infiltrated at different intervals after the shift. When the inhibitor-sensitive replication step was passed, the inhibition declined rapidly, thus providing evidence for the site of attack of the inhibitor in the virus replication process.

In a second series of experiments (Fig. 2A), synchrony was based on limiting the study to the first replication cycle. In this case the disks were punched out immediately after virus inoculation and treatment was started as described above.

Virus assay

PVX yield (concentration) was assayed serologically as described previously (Schuster et al., 1979), 72 h after the shift to permissive room temperature when using the DTI procedure (Fig. 2B) and 96 h after inoculation when the synchrony was based on the first replicative cycle (Fig. 2A). The number of dilution steps (twofold each) at which a precipitate did not occur served as an index. The average indices were calculated for each series and repetitions. The percentage of inhibition (I%) was determined as follows: $I\% = 100 - ({}^{AVC}\text{treatment}/{}^{AVC}\text{control} \times 100)$ (^{AVC}treatment is the average virus concentration index in the treated leaf disks; ^{AVC}control is the average virus concentration in the corresponding control disks). The significance of the observed differences was determined by Student's *t*-test.

Results and Discussion

As demonstrated in Fig. 1, AHPA 2-methylpropylester (AHPA-MP) efficiently inhibited PVX at low concentrations. A concentration of 10^{-5} mol·l^{-1} AHPA-MP gave an inhibition of approximately 75%, while a similar concentration of DHPA gave an inhibition of 8%. Complete inhibition of virus replication was obtained with 10^{-4} mol·l^{-1} AHPA-MP or $5 \cdot 10^{-4}$ mol·l^{-1} DHPA. AHPA-MP was not only superior to DHPA in its antiviral activity, but even at a concentration which totally inhibited PVX, it did not cause discernible damage to the leaf disks.

To obtain some information on the site of action of the two compounds in the PVX replicative cycle, the time-course of virus inhibition was followed without (Fig. 2A) or with (Fig. 2B) preincubation of the virus-infected leaf disks for 4 days at 5°C, with the minimum concentrations of compounds needed for full inhibition (Fig. 1). The time–response curves of virus inhibition were nearly identical for both compounds and independent of whether the leaves were incubated at permissive temperature only (Fig. 2A) or, using the DTI procedure, preincubated at 5°C for 4 days (Fig. 2B). Within the range of experimental error, these time (−response) curves were identical with that of cycloheximide which is considered to be an inhibitor of viral protein synthesis (Dawson and Schlegel, 1976).

Our data suggest that both DHPA and AHPA-MP owe their antiviral action to a similar mechanism, i.e. inhibition of a late event in the replicative cycle of PVX (viral mRNA synthesis and/or maturation, or early synthesis of viral protein). As both DHPA and AHPA-MP have been recognised as SAHase inhibitors (Votruba and Holý, 1980; Holý et al., 1985; De Clercq, 1987), the late event in the PVX replicative cycle inhibited by these compounds may well correspond to viral mRNA methylation. Further investigations are required to elucidate their precise mode of action.

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