CYTOPLASMIC POLYHEDROSIS VIRUS: RNA SYNTHESIZED IN VIVO AND IN VITRO IN INFECTED MIDGUT

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SUMMARY

RNA synthesized in vivo and in vitro in midgut infected with CPV is analyzed. Both single- and double-stranded RNA can be synthesized in vivo, but only single-stranded RNA in vitro.

The genome of cytoplasmic polyhedrosis virus (CPV) in insects is accepted as double-stranded RNA (1,2,3). However, irrespective of the extraction procedure employed, no intact genome molecules have been obtained (3,4). It was found that the extracted genome RNA fragments fell into a size distribution with two maxima, which was highly reproducible. These RNA fragments proved by a number of criteria to be double-stranded and not produced by random breaks in the molecule (2,3,4). During virus development it would be expected that virus-specific, single-stranded messenger RNA must result from the transcription of the parental genome in infected cells. It is the purpose of this communication to report on the detection of virus-specific single- and double-stranded RNA in infected cells. Further, the products which are synthesized in vitro by virus-induced RNA polymerase are characterized to identify the possible significance of RNAs produced in vivo.

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MATERIALS AND METHODS

Insects used in these experiments were Malacosoma disstria larvae. The virus strain used was M. disstria CPV, which is serologically different from that of Bombyx mori (5). Larvae in the 4th instar were infected by feeding polyhedra (6). After 6 days each infected larva which had reached the 5th instar was injected intrahemocoelically with 2 µl of an aqueous solution containing 1 µg of actinomycin D (7,8). The larvae were then re-injected with ^{3}H-uridine (2 µc/larva). Healthy larvae treated with both actinomycin D and ^{3}H-uridine were used as controls. The midguts were dissected out at different time intervals and assayed for radioactive RNA (8).

For in vitro assay, the midgut was dissected from infected insects and homogenized in TSM buffer (0.01M tris-chloride, pH 7.8 containing 0.25M sucrose and 0.005M $MgSO_4$) with 6 strokes in a Teflon-glass homogenizer in ice cold conditions. The homogenate was filtered through 4 layers of cheesecloth. The assay system contained, in a total volume of 5.0 ml (0.5-1.0 mg protein per ml), 10 µc ^{3}H-uridine and 2 µg of actinomycin D. The reaction mixture was incubated at 37°C for different time intervals. The sample (0.5 ml) withdrawn was treated with chilled trichloroacetic acid (TCA) in ice cold conditions. Acid-precipitable material, which includes the RNA, was collected on glass fiber filters, and the radioactivity was measured in a Packard Tri-Carb scintillation spectrometer (8).

The RNA was extracted by phenol procedures (9) from samples which were exposed to ^{3}H-uridine in vivo and in vitro, and the RNA was precipitated by addition of 3 volumes of cold ethanol and then dissolved in TK buffer (tris-chloride, pH 7.5, 0.025 M KCl) for sedimentation analysis (5-25% sucrose gradient) (9).

After sucrose gradient centrifugation, fractions were drop collected from the bottom of the centrifuge tube and an equal volume of 0.01 M $MgSO_4$ in TK buffer was added to each fraction. After determination of optical density at 260 mμ, each fraction was divided into two parts and RNase was added to one portion to a final concentration of 1 μg/ml. They were incubated for 30 min at 25 C. Yeast RNA (500 μg) and then an equal volume of cold 10% TCA was added. The radioactive materials were collected and assayed as described above.

For virion assembly *in vivo* and *in vitro* the same conditions were used as that described above. The virus was purified from 25 insect midguts and analyzed by using sucrose gradient centrifugation (10).

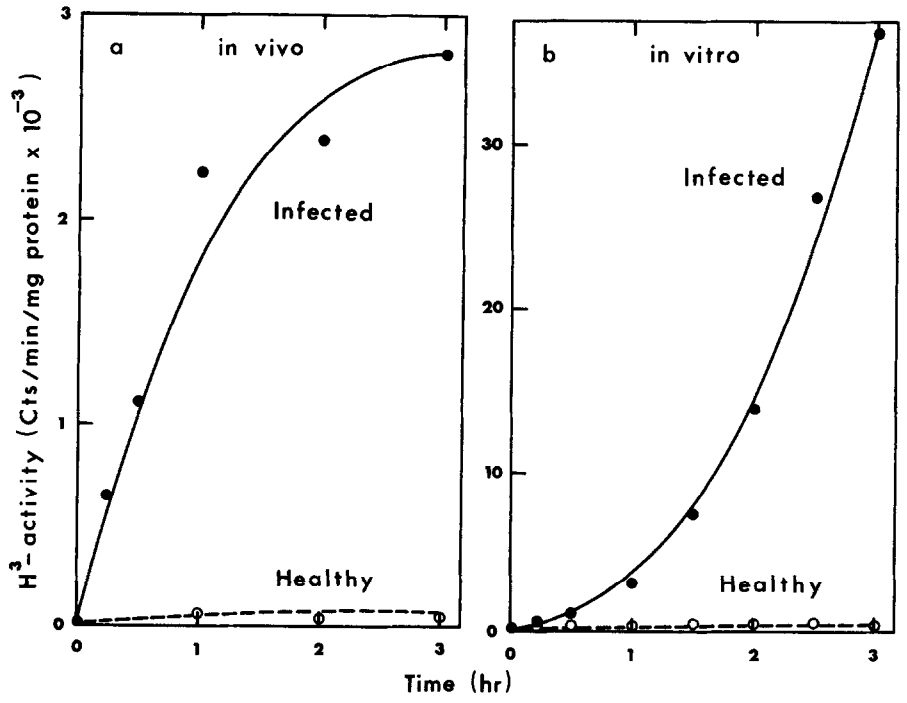

Fig. 1. RNA synthesis in healthy and infected midguts *in vivo* and *in vitro* in the presence of actinomycin D.

RESULTS AND DISCUSSION

Fig. 1 shows the *in vivo* and *in vitro* incorporation of ^{3}H-uridine into acid-precipitable materials in both healthy and infected midguts in the presence of actinomycin D. Concentration of actinomycin D used *in vivo* was 1 µg/larva. This amount of the drug does not decrease the virus yield (7), but reduces the amount of host cell RNA synthesis to very low levels within 1 hr (Fig. 1a Healthy). Double the amount of actinomycin D was added to the reaction mixture *in vitro*, and the RNA synthesis in healthy midgut was also almost abolished (Fig. 1b). Under these conditions, virus-induced RNA synthesis was detected both *in vivo* and *in vitro*. The activity *in vivo* reached a peak at 3 hr as shown in Fig. 1a while the reaction *in vitro* proceeded for at least 5 hr although there was a lag of about 1 hr before linear incorporation began.

After 3 hr labeling with ^{3}H-uridine in both conditions, RNA was extracted and analyzed using sucrose gradient centrifugation. The results are illustrated in Fig. 2. Three or four species of molecules labeled with tritium were present in the actinomycin D-treated, infected midgut *in vivo*. These newly synthesized RNAs sedimented with S values of 22, 15, 12 and 4. It is not known whether the 4 S RNA is virus induced (9). Single-stranded RNA is hydrolyzed by RNase whereas the double-stranded RNA is resistant to this treatment. When the RNA separated by sucrose gradient centrifugation was treated with RNase, the 22 S component and about 35% of the 15 S component were digested; the remainder of the 15 S and all of the 12 S were resistant to the enzyme. These results indicate that infected midgut cells contain both single- and double-stranded RNA.

The enzyme-resistant RNA consisted of two classes with sedimentation rates of 15 and 12 S. The viral genome RNA was also RNase resistant (3) having fragmented 15 and 12 S components as shown in Fig. 3, determined by using E. coli RNA as

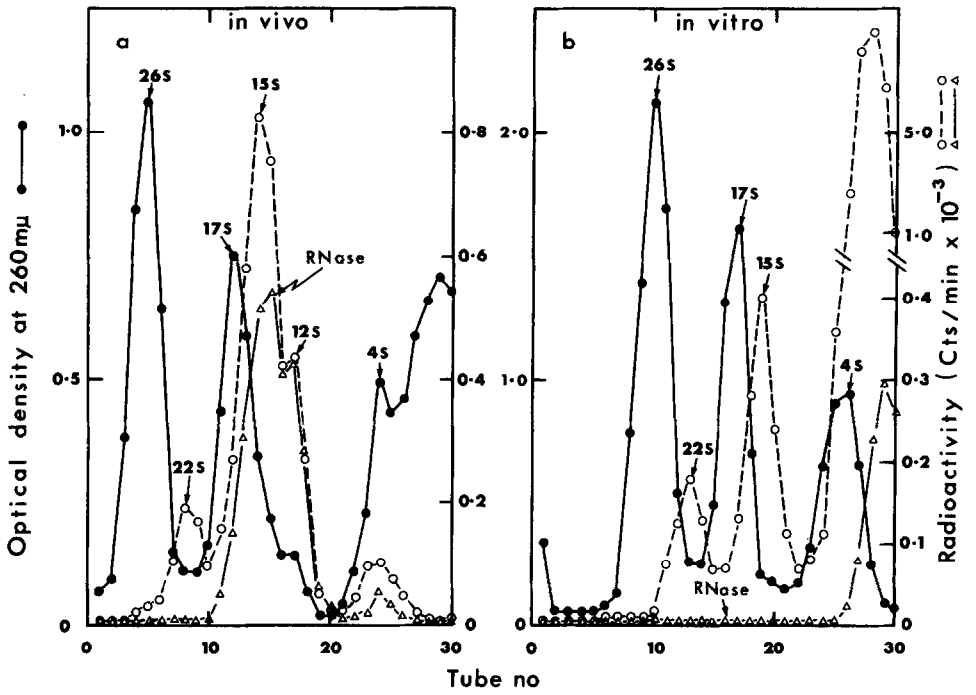

Fig. 2. Sucrose gradient (5-25%) sedimentation analysis of RNA synthesized by infected midgut cells in vivo and in vitro. a) Centrifuged at 30,000 rev/min for 16 hr in a SW 40 Ti rotor at 5C. b) at 23,000 rev/min for 20 hr.

a marker. These enzyme resistant RNAs can therefore be considered to represent viral progeny double-stranded RNA. Fig. 2b shows the sedimentation profiles of the products in vitro with midgut RNA as a marker. The three species of RNA enzymically synthesized sedimented with S values of 22, 15, and lower than 4 S. The 22 and 15 S molecules have the same sedimentation value as those extracted from infected midgut in vivo (Fig. 2a). The broad peaks lower than 4 S do not represent a single molecular

species, but are presumably viral genome directed ribopolymers. Exposure of the products to RNase degraded more than 90% of the radioactive material to acid-soluble form and this was evidently single-stranded RNA. However, the small amount of the enzyme-resistant material near the top of the gradient may be

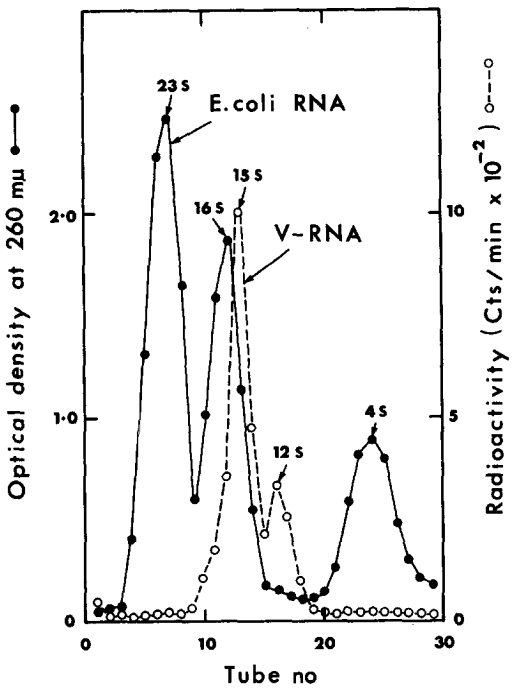

Fig. 3

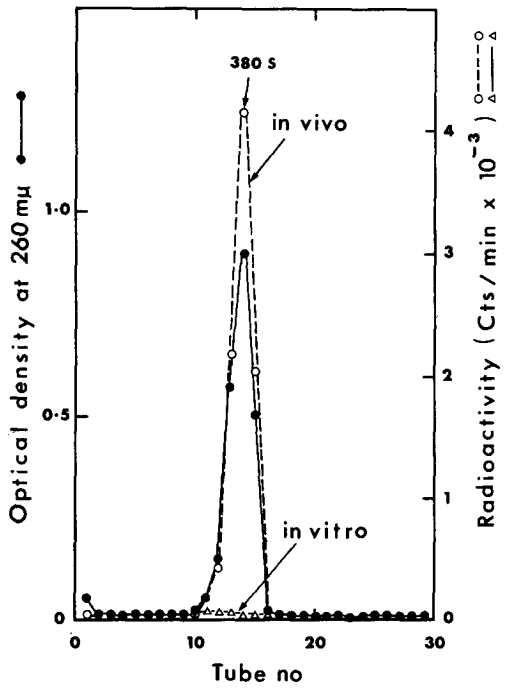

Fig. 4

Fig. 3. Sedimentation profile of viral RNA centrifuged together with E. coli RNA as markers. Centrifugation was performed at 30,000 rev/min for 16 hr.

Fig. 4. Examination of virus assembly in vivo and in vitro in infected midgut cells. Purified virus fractions were analyzed in sucrose gradient (10-40%) centrifugation. Optical density represents reference virus. Centrifugation was at 23,000 rev/min for 90 min at 5C.

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similar to the components that have been obtained from reovirus, i.e., enzyme-resistant single-stranded RNA (11). The results show clearly that although newly synthesized viral progeny RNA is detectable in vivo in the infected midgut, virus induced

single-stranded RNA can be synthesized *in vivo* and *in vitro*. In both conditions, the single-stranded molecules synthesized were considered to be identical in size as determined by sucrose gradient analysis. Unexpectedly, the relative sedimentation values of single- and double-stranded RNA synthesized, except the lower components, are very similar to those reported for reovirus studies (12-16) although the fragmentation of genome RNA is different. It is tempting to speculate that if CPV has a replication process similar to reovirus (17), the single-stranded RNA synthesized in the infected midguts probably represents virus messenger RNA transcribed from viral genome.

As described above, double-stranded RNA is synthesized in the infected midgut and it is suggested that this is viral progeny RNA. If double-stranded RNA occurred only in the subviral particle or virion as in reovirus (14), it would not be expected that the CPV virion could be assembled in the *in vitro* conditions used since no double-stranded RNA was detected. To prove whether or not the virion could be assembled, the isolation of newly assembled virion was attempted *in vitro*. Virion synthesis *in vitro* was unsuccessful (Fig. 4). It is apparent that virion formation in cell free fraction is difficult and requires appropriate conditions, such as the intact virus-synthesizing factories, viroplasm (18).

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