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Isolation and reconstitution of the RNA replicase of the cytoplasmic polyhedrosis virus of silkworm, *Bombyx mori*

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Summary. After irradiation of the virus particles of CPV, the RNA replicase associated with the virion was isolated in the form of a genome-replicase complex with DEAE-Sephadex A-25 chromatography. This complex was then treated with Triton X-100 and purified by phosphocellulose column chromatography. The RNA replicase reconstituted with the double-stranded RNA of CPV showed both the enzyme activity of RNA polymerase and methyltransferase. The single-stranded RNA could not serve as the template for the RNA replicase. The role of the RNA replicase of CPV is discussed.

Key words: RNA replicase – Cytoplasmic polyhedrosis virus – *Bombyx mori*

Introduction

The cytoplasmic polyhedrosis virus (CPV) of the silkworm is a typical prototype of the double-stranded RNA virus group Payne and Mertens (1983). The virus particles of CPV contain 10 segments of the double-stranded RNA genome and RNA replicases. The RNA replicase and methyltransferase have been isolated in the form of genome-replicase complex Dai Ren-Ming et al. (1982): the latter consists of three kinds of protein subunits Dai Ren Ming (submitted). The isolation and reconstitution of the RNA replicase of the CPV of silkworm will be described in this paper.

Materials and methods

The materials used were (^{3}H-CH$_3$)-S-adenosyl-L-methionine (72 Ci/mole) (Amersham), DEAE-Sephadex A-25 (Pharmacia), triton X-100 (ROTH), phosphocellulose p-11 (Whatman), (5,6-^{3}H)-UTP (17 Ci/mmole) (Shanghai Institute of Nuclear Research), and UTP, ATP, GTP, CTP phosphoenolepyruvic acid and pyruvic acid kinase (Dong-Fen Biochemical Factory). The S-adenosyl-L-methionine was prepared by Cantoni's method (Cantoni 1975). The CPV of the silkworm was purified according to the method of Wu Ai-Zhen et al. (1981).

Isolation of the CPV replicase

The preparation of CPV (15 ml, $A_{260\,nm}=47$) was placed in a Petri dish to a depth of 2–3 mm and illuminated with an ultraviolet lamp (BIG-69 coil type sterilizing lamp, 10 W, 2,537 Å) at a distance of 10 cm above the dish, for 20 min, with occasional stirring. After irradiation, the CPV preparation was applied to a DEAE-Sephadex A-25 column (Cl^- form, 0.5×30 cm) and washed with 50 ml of NaCl in 0.5 M to remove impurities. The genome-replicase complex was eluted with 1.0 M NaCl and the fraction was pooled and dialysed against cold redistilled water to remove the NaCl. The genome-replicase complex was obtained in 15 ml ($A_{260\,nm}=5.85$).

The genome-replicase complex (15 ml) was dissociated with Triton X-100 at a concentration of 0.5%, at 37 °C for 2 h, and then applied to the phosphocellulose column (K^+ form, 1×8 cm, pretreated with a 0.01 M phosphate buffer, pH 7.8). The RNA replicase was eluted with a 0.01 M phosphate buffer, pH 7.8, containing 0.5 M KCl, and dialyzed against the phosphate buffer in the presence of 2-mercaptoethanol (0.1%) to remove the Triton X-100 and KCl. It was then concentrated into a small volume at 4 °C.

Preparation of CPV double stranded RNA

The preparation of CPV was extracted 5 times with a phenol solution (90%). The water phase was collected and washed with ether (without peroxide) to remove the remaining phenol. The double-stranded RNA solution obtained was 2.35 mg/ml, the ratio of $A_{260\,nm}/A_{280\,nm}=2.0$ and $A_{260\,nm}/A_{230\,nm}=2.24$.

Reconstitution of the replicase and of the double-stranded CPV RNA

The reaction system (500 µl) consisted of 2.75 mM ATP, 1.66 mM GTP, 1.67 mM CTP, 1.71 mM UTP, 0.5 mM phos-

phoenolepyruvic acid, 0.16 mg bentonite, 35 µl S-adenosyl-L-methionine (1.12 µmole/ml), 0.16 mg pyruvic kinase, 80 µl Tris buffer (pH 8.0, 1.0 M), 50 µl $MgCl_2$ (0.05 M), 100 µl replicase of CPV of silkworm and 58 µg of double-stranded CPV RNA. The control system was the same as the reaction system minus the double-stranded RNA. The UTP was replaced by the ^{3}H-UTP in the system for determination of the activity of the RNA polymerase. The SAM was replaced by ^{3}H-CH_3-SAM (20 µCi) in the system for determination of the activity of methyltransferase Sun Yu-Kun (1981).

Results

Isolation of the CPV replicase

After illumination of the preparation of CPV with ultraviolet light, the preparation was applied to the DEAE-Sephadex A-25 column and washed with different concentrations of NaCl. The replicase was subsequently isolated in the form of the genome-replicase complex (Fig. 1).

As shown in Table 1 the characteristics of the UV absorption of the genome-replicase complex is different from that of the dsRNA, the ratio of A_{260nm}/A_{280nm} being lower than that of the dsRNA of CPV because the genome-replicase complex contains proteins. The replicase was separated from the genome-enzyme complex by Triton X-100 treatment and the column chromatography of phosphocellulose showed a typical protein UV absorption (Fig. 2).

Reconstitution of the replicase and double-stranded RNA of CPV

As shown in Figs. 3 and 4 when the replicase reconstituted with replicase and the double-stranded RNA of CPV, the enzyme activities of RNA polymerase and methyltransferase were very much enhanced. If the double-stranded RNA in the reaction system was replaced by the single stranded RNA of TMV, the replicase showed no RNA polymerase activity.

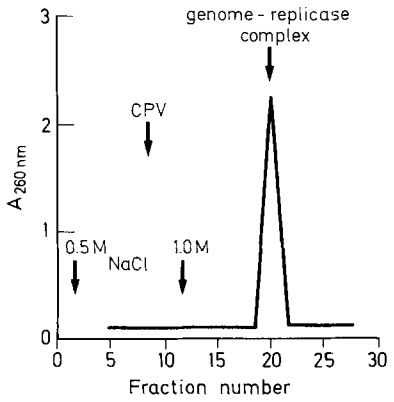

Fig. 1. Isolation of the genome-replicase complex

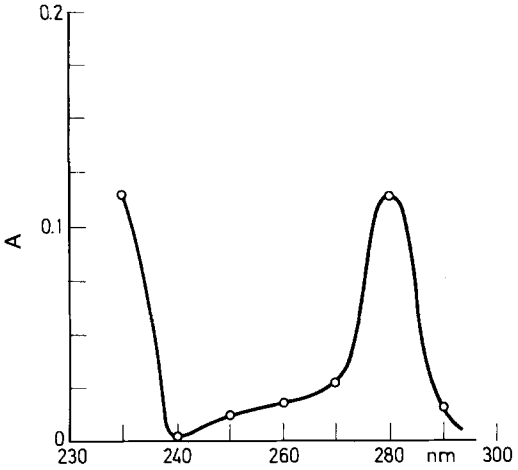

Fig. 2. UV absorption spectrum of the CPV replicase

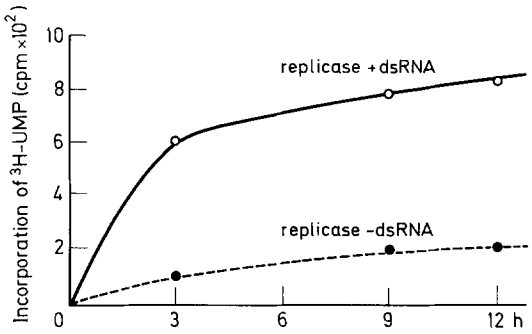

Fig. 3. Activity of RNA polymerase after reconstitution of the replicase with CPV double-stranded RNA

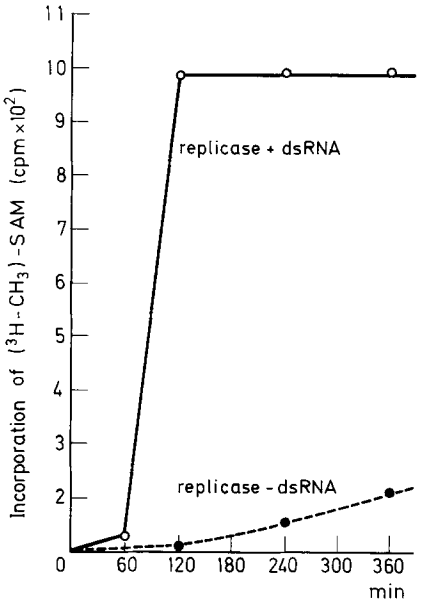

Fig. 4. Activity of methyltransferase after reconstitution of the replicase with CPV double-stranded RNA

Table 1. Characteristics of the genome-replicase complex of CPV

	A_{260}/A_{280}	A_{260}/A_{242}	A_{260}/A_{230}
CPV	1.6–1.7	1.2	
dsRNA of CPV	2.0		2.24
Genome-replicase of CPV	2.0		1.8

Discussion

The RNA replicase in the virion of CPV of the silkworm is tightly bound to each segment of the double stranded RNA genome. Even through the dissociation of the virus particles' CPV by DEAE-Sephadex chromatography and polyacrylamide gel electrophoresis, the RNA replicase could not be separated from the genomes. In order to study the function of the RNA replicase of CPV, it is essential to isolate the enzymes in the active form. The RNA replicase of CPV contains three kinds of protein subunits. The RNA replicase of CPV has been isolated by the treatment of the complex with Triton X-100 Wu Ai-Zhen (1981) and phosphocellulose column chromatography. The replicase reconstituted with double-stranded RNA of CPV shows enzyme activities of both RNA polymerase and methyltransferase. The single-stranded RNA could not serve as the template for the RNA replicase. We consider that the RNA replicase associated with the virus particles of CPV plays a role in the transcription of double-stranded RNA to form the mRNAs, whereas the synthesis of the double-stranded RNA of CPV may be catalyzed by other RNA replicases in CPV infected cells.

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Reference

Cantoni GL (1957) S-adenosylmethionine (SMe). Biochem Prep 5:58–61

Dai Ren Ming, Wu Ai Zhen, Shen Xue Ren, Sun Yu Kun (1982) Isolation of genome-enzyme complex from cytoplasmic polyhedrosis virus of silkworm, *Bombyx mori.* Sci Sin 25:29–35

Dai Ren Ming, Wu Ai Zhen, Shen Xue Ren, Sun Yu Kun (1985) The protein subunits of the double stranded RNA dependent RNA polymerase and methyltransferase of the cytoplasmic polyhedrosis virus of Silkworm, *Bombyxx mori.* Sci Sin (submitted)

Payne CC, Mertens PPC (1983) Cytoplasmic polyhedrosis viruses. In: Joklik WK (ed) The reoviridae. Plenum, New York, pp 424–504

Sun Yu Kun, Wu Ai Zhen, Dai Ren Ming, Shen Xue Ren (1981) Synthesis of structural protein in a cell free system directed by silkworm cytoplasmic polyhedrosis virus mRNA synthesized in vitro. Sci Sin 24:684–691

Wu Ai Zhen, Dai Ren Ming, Shen Xue Ren, Sun Yu Kun: (1981) (^{3}H-methyl)-methionine as possible methyl donor for formation of 5-terminus of in vitro synthesized mRNA of cytoplasmic polyhedrosis virus of silkworm. *Bombyx mori.* Sci Sin 24:1737–1742

Wu Ai Zhen, Dai Ren Ming, Shen Xue Ren, Sun Yu Kun (1981) Relationship between the RNA polymerase and infectivity of cytoplasmic polyhedrosis virus of silkworm, *Bombyx mori.* Acta Microbiol Sin 21:73–76