203. Reconstitution of Tobacco Mosaic Virus *in vitro*

Inhibition by Coat Proteins of Other Strains

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Typical tobacco mosaic virus (TMV) particles have been reconstituted from their two components; the viral coat protein and the viral nucleic acid.^{1),2)} The preparation of mixed viral particles containing the RNA from one strain of TMV and the coat protein from another strain has been a useful tool in the study of the genetic and biologic roles of these two viral components, as reported by Fraenkel-Conrat and Singer.³⁾ Holoubek⁴⁾ showed that the reconstitution of the common TMV protein with the RNA of chemically produced mutants proceeded similarly as compared with the common TMV-RNA. He also noted that HR-RNA reconstituted slightly less well than did the common TMV-RNA. In this paper, we have analyzed the infectivity of a product viral particle which was composed of RNA from a common strain and a protein or a mixture of proteins from different strains of TMV. Of greater interest, also, was the inhibition of common TMV reconstitution caused by coat proteins from other strains.

Materials and methods. The viruses of TMV-OM (Japanese common strain),⁵⁾ TMV-O (Japanese common strain)⁶⁾ and TMV-Vulgare (German common strain)⁷⁾ were prepared from systemically infected leaves of tobacco (*N. tabacum* var. Bright Yellow). TMV-L (Japanese tomato strain)⁸⁾ and TMV-HR (Holmes ribgrass strain)⁹⁾ were also prepared from tobacco (*N. tabacum* var. Xanthi). CGMMV-C (cucumber green mottle mosaic virus, cucumber strain)¹⁰⁾ and CGMMV-W (cucumber green mottle mosaic virus, watermelon strain)¹¹⁾ were cultured in cucumber (*Cucumis sativus* L.) and TYMV (turnip yellow mosaic virus)¹²⁾ was cultured in chinese cabbage (*Brassica chinensis* L.). There are various views of the serological relation between TMV and CGMMV,¹³⁾⁻¹⁶⁾ but CGMMV-C and W we used reacted with anti-TMV-OM.¹⁷⁾

All virus preparations were purified by differential centrifugation. Proteins were prepared from these strains by the acetic acid method.¹⁸⁾ TYMV was treated with iodoacetate to solubilize its protein prior to acetic acid treatment.¹⁹⁾ RNA was prepared from

TMV-OM by the phenol method.²⁰⁾ P³²-labelled TMV-RNA was prepared from P³²-labelled TMV-OM.

The reconstitution was carried out according to the method of Fraenkel-Conrat and Singer²¹⁾ with a slight modification. The reaction mixture consisted of 10 μ g of TMV-RNA and 300 μ g of protein in 0.1 M pyrophosphate buffer, pH 7.2, in 0.3 ml final volume. Incubation was at 30°C for 5 hrs. The mixture was diluted about 1,000-fold with 0.01 M phosphate buffer, pH 7.0, in ice water and inoculated to tobacco plant (*N. tabacum* var. Xanthi nc) for local lesion assay by the half leaf method. Each assay based on 8–12 half leaves.

Experiments on the sucrose density gradient (5–20% sucrose linear gradient in 0.1 M pyrophosphate buffer, pH 7.2) were carried out with the sample of the incubated mixture using the same method as above, but scaling up 10-fold. The tubes were centrifuged in a RPS-25 rotor (Hitachi Model 55P) for 140 mins at 24,000 r.p.m. for the reconstituted particle analysis and for 16 hrs at 24,000 r.p.m. for the RNA analysis. After centrifugation, the gradients were fractionated through the bottom and the O.D. at 260 m μ and the radioactivity was measured for each tube.

Results and discussion. 1) *Reconstitution of the common TMV-RNA with the coat proteins of other TMV strains.*

The results of reconstitution of RNA from OM strain with

Table I. Reconstitution of common TMV-RNA with various coat proteins

Proteins	Infectivity
	Reconstitution %
TMV-OM	100
TMV-O	113
TMV-Vulgare	98
TMV-L	8
TMV-HR	29
CGMMV-W	99
CGMMV-C	12
lysozyme	0
TYMV	0

Assays were carried out as described in *Materials and methods*. In all cases 300 μ g of protein was mixed with 10 μ g of RNA. The diluted reaction mixture was inoculated on a tobacco half leaf. As control, the reaction mixture containing TMV-OM protein was inoculated onto another half leaf. The reconstitution percentage is expressed as the mean value of the ratio of the number of local lesion which appeared 3 days after inoculation on the half leaf to the number of local lesion on the control half leaf.

proteins from other TMV strains are summarized in Table I. The coat proteins which belong to common strains, Vulgare and O, could reconstitute with OM-RNA as well as OM-protein. The amino acid sequences of these three common strains were identical except one or two residues.^{5),6)} On the other hand, proteins from other strains resulted in varying degrees of yield of reconstituted viral particles. Yields upon reconstitution of TMV-L, HR, CGMMV-W and C were 8, 29, 99 and 12% respectively. They differ in total amino acid composition from the common strain in 8,²¹⁾ 17,²²⁾ 17²³⁾ and 30²⁴⁾ residues respectively. Although no general consistency between the groups, suggested by chemical data and ability to reconstitute, could be demonstrated, these results suggest that a specific structural relation of protein to RNA must be the main factor directing reconstitution. In the homologous system, common-protein and common-RNA, the yield of reconstitution was the best. Proteins unrelated to TMV-protein, such as TYMV-protein and lysozyme, did not reconstitute with TMV-RNA.

2) *Inhibition of common TMV reconstitution by coat proteins from other strains.*

By the addition of proteins from other strains to the control reaction mixture containing RNA and protein of OM strain, inhibition of reconstitution was observed in the case of TMV-L, HR and CGMMV-W protein as shown in Table II. The more the protein of

Table II. Inhibition of common TMV reconstitution with various other coat proteins

Proteins	Infectivity	
	Added amount of proteins	
	75 μ g	300 μ g
TMV-OM	146	142
TMV-O	111	127
TMV-Vulgare	159	134
TMV-L	56	16
TMV-HR	49	20
CGMMV-W	7	10
CGMMV-C	103	108
lysozyme	112	89
TYMV	105	103

To the reaction mixture containing 10 μ g of OM-RNA and 300 μ g of OM-protein, 75 or 300 μ g of excess protein indicated was added and incubated. Assay was carried out as described in Table I. Infectivity of each preparation was compared with that of the standard reaction mixture, 10 μ g of OM-RNA and 300 μ g of OM-protein.

Fig. 1. Separation on a linear sucrose gradient (5-20 %) of reconstituted TMA preparation.

●—● O. D. at 260 mμ. ×---× radioactivity.

(A) 100 μg of RNA (OM) and 3.0 mg of protein (OM).

(B) 100 μg of RNA (OM), 3.0 mg of protein (OM) and 3.0 mg of protein (CGMMV-W).

(C) 100 μg of RNA (OM) and 3.0 mg of protein (CGMMV-W).

the other strain was added to the protein of the OM strain, the more the reconstitution was inhibited. Such an inhibiting effect was not observed in the case of protein of the common strain and proteins different from the TMV-protein such as TYMV-protein and lysozyme.

As a typical model of the inhibition, the inhibitory reaction by CGMMV-W protein was analyzed by a density gradient centrifugation method. As mentioned above, a highly infective preparation can be obtained with CGMMV-W protein as well as OM-protein (Table I). The same result was shown by the density gradient experiments, indicating that the formation of a sedimentable particle could be obtained with CGMMV-W protein as well as with OM-protein (Fig. 1, A and C). However, few reconstituted particles were obtained when both proteins were present in the reaction mixture at the same time (Fig. 1, B). This result indicated that the inhibition of reconstitution by CGMMV-W protein was due to the inhibition of the formation of infective particles, and not the formation of a non-specific aggregate mixture having no infectivity. Similar results were obtained in the case of inhibition by TMV-L protein.

What actually happens in the course of inhibition is not clear. We would like to propose a specific structural relation of protein to RNA at the particular site on the RNA molecule where reconstitution starts. At the beginning of the reconstitution reaction, if two kinds of protein having similar but not the same structural specificity are present at the same time, the specific interaction between the RNA and a particular protein will be interfered with another reacting protein.

We know the well-established phenomenon of interference or cross protection occurs among some strains of a plant virus. Inhibition of the reconstitution of a TMV strain by proteins from other TMV strains resembles the interference *in vivo*. As a possible mechanisms of interference between strains of a plant virus, such an inhibitory reactions is not unimaginable.

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