The Composition of Tomato Bushy Stunt Virus from Prunus avium

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ABSTRACT

The composition of two strains of tomato bushy stunt virus, the type strain (TBSV-T) and one isolated from Windsor sweet cherry (TBSV-P), was 17% RNA and 83% protein based on analyses of recovered nucleotides and amino acids. The molar percentages of the nucleotides in the RNA of TBSV-P were 27% adenylic acid, 28% guanylic acid, 20% cytidylic acid, and 25% uridylic acid; these were not significantly different from the values for TBSV-T.

Consideration of the size, structure, and amino-acid analyses of TBSV indicated that the virus protein subunit was composed of approximately 290 amino-acid residues. The amino-acid compositions of the two strains were similar, and differences between them represented only seven amino acid exchanges. However, the serological properties of the two strains differed greatly. The significance of this apparent anomaly is discussed. Phytopathology 60:454-456.

When de Fremery & Knight (3) analysed three strains of tomato bushy stunt virus (TBSV) selected for their widely differing effects on *Datura stramonium* L., they found no significant differences in their composition. However, Allen & Davidson (2) isolated a strain of TBSV from *Prunus avium* L. variety Windsor (TBSV-P) that differed serologically from the type strain (TBSV-T). Measurement of the content of strain-specific antibodies in antisera indicated that the TBSV-P and TBSV-T strains differed greatly in serological properties (1). This study compares the chemical composition of these two strains of TBSV.

MATERIALS AND METHODS.—Rutgers tomato seedlings (*Lycopersicon esculentum* Mill.) were inoculated with cultures of TBSV-P or TBSV-T obtained from W. R. Allen, Canada Department of Agriculture, Research Station, Vineland, Ontario. Three weeks later, the infected plants were harvested and ground in a meat grinder. EDTA buffer at pH 6.0 and 0.1 M was added to the expressed sap to a concentration of 0.01 M. The sap was clarified by low-speed centrifugation at 10,000 rpm for 20 min in a GSA rotor of a Servall refrigerated centrifuge. The virus was sedimented at 28,000 rpm for 90 min (No. 30 rotor, Spinco Model L ultracentrifuge), resuspended in 0.01 M Tris [tris (hydroxymethyl) amino methane]-chloride buffer pH 7.4 and clarified at 10,000 rpm for 15 min in the No. 30 rotor. This cycle of differential ultracentrifugation yielded brown opalescent preparations which were passed through a 100 × 25-cm column of Bio-Gel A-5 m in 0.01 M Tris-chloride buffer pH 7.4 at 15 ml/hr. The fractions containing virus eluted from this column were given another cycle of differential centrifugation. Examination of the resulting colorless opalescent preparations at concentrations of 7 mg/ml in the analytical ultracentrifuge and by scanning density-gradient columns in an ISCO density gradient ultraviolet monitor showed the presence of a single component. Virus yields were from 50 to 100 mg/kg of infected tissue.

Amino acids and nucleotides of the two viruses were analysed as reported previously (7). Nucleic acid and protein were separated by mixing 4 ml of a preparation containing approximately 7 mg/ml of virus with 4 ml of 2 N HCl. This mixture was kept at room temperature for 18 hr; the denatured protein was then pelleted by centrifugation and washed once with 1 N HCl. The supernatant and washing were pooled, hydrolyzed in boiling water for 1 hr, and dried, and purine bases and pyrimidine nucleotides were determined by paper chromatography.

The protein pellets were dissolved in 12 N HCl, and aliquots were pipetted into three tubes. The tubes were evacuated, sealed, and hydrolyzed in an oven at 107 C for 12, 24, and 72 hr. After hydrolysis, the excess HCl was removed in a flash evaporator, and the samples were dissolved in 8 ml of citrate buffer, pH 2.2. Aliquots of 1 ml were analyzed with a Spinco amino acid analyzer. Cysteine determinations were made on separate samples of virus protein oxidized with performic acid before hydrolysis. The tryptophan content was determined by the reaction of tryptophan in undegraded virus with *p*-amino-benzaldehyde (6). The amount of virus protein in these preparations was determined by amino acid analysis of an aliquot of each.

RESULTS AND DISCUSSION.—The average composition of recovered nucleotides in three analyses in mole per cent were:

	Guanylic acid	Adenylic acid	Cytidylic acid	Uridylic acid
TBSV-P	28.0	26.9	19.8	25.3
TBSV-T	27.3	27.5	20.2	25.0

These analyses were not significantly different from each other or from the analysis reported by de Fremery & Knight (3). The percentage RNA in the virus, calculated from the nucleotides and amino acids recovered, was 16.8 and 17.4 for TBSV-P and TBSV-T, respectively.

The analytical data for the amino acid composition of the two strains are presented in Table 1. The values obtained for the 12-hr, 24-hr, and 72-hr hydrolyzates are included in the average unless otherwise noted. The amounts of threonine and serine were calculated by extrapolating the 12-hr and 24-hr hydrolyzate values

TABLE 1. Nanomoles of amino acids recovered from preparations of the *Prunus* (TBSV-P) and type (TBSV-T) strains of tomato bushy stunt virus

Amino acid	TBSV-P	TBSV-T
Ala	628[a]	703
Arg	334	363
Asp	738	803
Cys[b]	72	53
Glu	393	427
Gly	695	748
His	73	89
Ile[c]	244	252
Leu[c]	817	767
Lys	222	247
Met	47	80
Phe	235	296
Pro	270	277
Ser[d]	529	598
Thr[d]	748	878
Try[e]	30	30
Tyr	196	222
Val[c]	727	773

[a] Average of at least two analyses of each of the 12-, 24- and 72-hr hydrolysates.
[b] Determined as cysteic acid from performic acid-oxidized protein.
[c] Only 72-hr hydrolysis analyses included in average.
[d] Extrapolated to zero hydrolysis time.
[e] Determined by the colorimetric analysis of Spies & Chambers (6).

to zero hydrolysis time. Valine and isoleucine yielded significantly greater recoveries from 72-hr hydrolyzates, while leucine remained fairly constant.

Since TBSV has a molecular wt of 8.9×10^6 (5), is composed of 83% protein (3), and probably has 240 protein subunits/virus particle (4), the molecular wt of each protein subunit should be 30,800. The molar ratios of amino acid in Table 1 for TBSV-P and TBSV-T were multiplied by 416 and 384, respectively, to yield a total of 290 amino acids for a protein subunit molecular wt of approximately 30,800 (Table 2). For comparison, the data of de Fremery & Knight (3) were treated in a similar manner.

TABLE 2. Amino acid residues in a protein subunit of the *Prunus* (TBSV-P), the type (TBSV-T), and the BS 3 strains of tomato bushy stunt virus

Amino Acid	TBSV-P	TBSV-T	TBSV strain BS 3[a]
Ala	26.1	27.0	26.2
Arg	13.9	13.9	14.5
Asp	30.8	30.8	31.7
Cys	3.0	2.0	2.1
Glu	16.4	16.4	15.3
Gly	29.0	28.7	26.8
His	3.0	3.4	3.4
Ile	10.2	9.7	9.4
Leu	34.1	29.4	31.2
Lys	9.2	9.4	9.6
Met	1.9	3.1	2.1
Phe	9.8	11.4	9.9
Pro	11.3	10.6	11.5
Ser	22.1	22.9	25.5
Thr	31.2	33.7	31.7
Try	1.2	1.2	1.3
Tyr	8.2	8.4	7.6
Val	30.4	29.6	29.1
Total	291.8	291.6	288.9
Total integer residues	290	291	290

[a] de Fremery & Knight (3).

Comparison of the integer values of the number of residues of each amino acid in the TBSV-P and TBSV-T strains in Table 2 indicate differences of 1 alanine, 1 cysteine, 5 leucine, 1 methionine, 1 phenylalanine, 1 serine, and 3 threonine residues. It is doubtful that these differences, representing seven exchanges, are all significant. If differences of less than 5% in residue values are ignored, there are only four exchanges. The analysis of the BS 3 strains (3) is similar to the other strains.

Comparison of the differences in amino acid composition and serological properties between the TBSV strains and between the southern bean mosaic virus (SBMV) strains is interesting. A method of comparing the serological properties of strains is to determine the titer or dilution end point of an antiserum by titration against both homologous and heterologous strains. The cross-reactive antibody ratio, determined by dividing the reciprocal of the heterologous titer by the reciprocal of the homologous titer, is a measure of the similarity of the two strains. However, values of the cross-reactive antibody ratio for a given pair of strains vary considerably with the time of bleeding and the amount of antigen used to induce the antibodies (1, 9). Nevertheless, the content of cross-reactive antibodies in antisera taken from a rabbit 6 weeks or more after injection of one or more mg of antigen remains fairly constant. The cross-reactive antibody ratios of antisera to TBSV strains are approximately 0.20 to 0.40 (3, *unpublished results*), but those of antisera to SBMV strains are 1.00 by this method (9). Strain-specific antibody could be detected only by other methods in this study. These results indicate great differences in the serological properties of the TBSV strains, and smaller differences in the serological properties of the SBMV strains. However, the differences in amino acid composition between the SBMV strains (8) were at least 21 amino acid exchanges, in contrast to 7 for the TBSV strains.

The lack of positive correlation between differences in amino acid composition and differences in serological properties is not surprising. The serological properties of a virus particle are determined by the sequence of amino acids on the surface of the particle and their conformation. Hence, the numbers of exchanges are not so important as their positions in the virus particle. A comparison of amino acid compositions does not indicate the nature or position of amino acid exchanges or their effect on conformation. For example, analyses of the proteins of some strains of tobacco mosaic virus showed fewer differences than analyses of separated tryptic peptides (7). The fortuitous compensation of complementary amino-acid exchanges accounts for this result. Besides these chemical considerations, little is known about the mechanism of induction of cross-reactive and strain-specific antibodies.

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