Partial Nucleotide Sequences for Three Unique T_1 Ribonuclease Fragments of Tobacco Mosaic Virus Ribonucleic Acid*

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ABSTRACT: Three fragments, having chain lengths of 26, 26, and 70 nucleotides, have been isolated from T_1 ribonuclease digests of tobacco mosaic virus ribonucleic acid (TMV RNA). These fragments appear to be unique in the TMV RNA molecule. When digested with pancreatic ribonuclease, the three fragments were found to yield the following products: 12 Up, 3 ApUp, 4 $(Ap)_2Up$, 1 Cp, 6 ApCp, 6 $(Ap)_2Cp$, 2 $(Ap)_3Cp$, 1 Gp; 2 Up, 2 ApUp, 1 $(Ap)_2Up$, 1 $(Ap)_3Up$, 4 Cp, 2 ApCp, 1 $(Ap)_3Cp$, 1 Gp; 5 Up, 2 ApUp, 1 $(Ap)_2Up$, 1 $(Ap)_3Up$, 2 Cp, 2 ApCp, 1 $(Ap)_2Cp$, 1 Gp. None of the three fragments can be a portion of the gene which codes for the TMV coat protein. The similarity of the pancreatic ribonuclease digestion products of the two 26-mers suggest the possibility of genetic duplication in TMV RNA.

Most of the difficulties encountered in the sequence analysis of virus RNA stem from the large sizes of these molecules. Attempts to limit the analysis to smaller, specific segments have been successful in Qβ RNA, where the sequence of a portion approximately 160 bases long has been determined (Billeter *et al.*, 1969), and in R-17 RNA, where the sequence of part of the protein coat cistron has been established (Adams *et al.*, 1969). The stripping of part of the protein coat from TMV has been suggested as a means for exposing specific portions of TMV RNA (May and Knight, 1965), and has been used in the location of a local lesion factor and the coat protein cistron of TMV (Kado and Knight, 1966, 1968).

We have used the partially stripped virus to carry out sequence analysis of the exposed RNA, and in particular, to locate three unique long-chain oligonucleotides from T_1 RNase digests of TMV RNA (Mandeles, 1968). These fragments were designated as the Ψ_1-mer, Ψ_2-mer, and Ω-mer, and it was shown that the 3′-linked end of the Ω-mer may be as little as 180 nucleotides away from the 5′-linked end of TMV RNA. The sequence analysis of the Ψ-mers and the Ω-mer is made difficult by the fact that, of the two readily available specific nucleases, they are substrates for only one, pancreatic RNase. Nevertheless, the chemical and physical studies reported here provide information on the uniqueness, chain length, and partial sequences of these oligonucleotides.

Materials and Methods

Preparation of TMV RNA. Tobacco mosaic virus was isolated from infected tobacco leaves and TMV RNA was isolated from the virus by the phenol extraction method as described before (Mandeles and Bruening, 1968).

Hydrolysis of TMV RNA with T_1 RNase. TMV RNA, in 200-mg batches, was hydrolyzed to completion with RNase T_1 (Calbiochem). The RNA, as the ethanol precipitate, was dissolved in distilled water to give a solution containing 3–4 mg/ml of RNA. This solution was centrifuged at 17,000*g*, 4°, for 30 min to remove the Macaloid, an anionic clay, introduced during the RNA preparation to inhibit nuclease action. The supernatant was collected and recentrifuged as above until the resulting supernatant was free of turbidity. The hydrolysis was performed at pH 7.8, 40° in the presence of 15,000 units (Egami and Takahashi, 1961) of T_1 RNase. Two-thirds of the enzyme was added initially and the remainder was added 2 hr later. The reaction, which was followed in a pH-Stat, reached completion after 6–8 hr. The end point as indicated by the pH-Stat was routinely found to be 92–97% of the value predicted from the ultraviolet absorbance of the RNA.

Hydrolysis of T_1 RNase Fragments with Pancreatic RNase. A sample of the T_1 RNase fragment to be hydrolyzed was dissolved in distilled water to give a final concentration of roughly 5 mg/ml. Potassium phosphate buffer (1.0 M, pH 7.9) and pancreatic RNase (Worthington three-times crystallized) were added to give a reaction mixture with a buffer concentration of 0.025 M and an enzyme:substrate ratio of 1:20 mg. The digestion was allowed to proceed for 3.5 hr at 37°.

Chromatography. Column chromatography was performed with DEAE-Sephadex A-25 (Pharmacia). The 7 M urea used in the chromatographic work was made with reagent grade urea and was treated with charcoal to remove any ultraviolet-absorbing impurities. The procedures used for effecting separations upon the basis of chain length and nucleotide composition have been described elsewhere (Rushizky *et al.*, 1964). The procedure used to separate the products of pancreatic RNase digestion of T_1 RNase fragments was performed on a DEAE-Sephadex column having a bed volume of 4 ml (0.4 × 35 cm). The column was loaded with 0.3–2.0 mg of hydrolysate and then washed with 30 ml of 0.001 M HCl at a flow rate of 15 ml/hr before initiating a pH gradient. Although no material is eluted from the column, this step is a necessary

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TABLE I: Yields Obtained for Various Preparative Procedures.

Amount of Starting Material	Yield of Product
2.4 kg of infected tobacco leaves	2–4 g of TMV
5 g of TMV	200 mg of TMV RNA
200 mg of TMV RNA	55 $A_{260}^{cm^2}$ of Ω-mer[a]
	45 $A_{260}^{cm^2}$ of Ψ-mers
	(yield after rechromatography)
	50 $A_{260}^{cm^2}$ of Ω-mer
	15 $A_{260}^{cm^2}$ of Ψ-mer
	15 $A_{260}^{cm^2}$ of Ψ_2-mer

[a] 1.0 mg is approximately equivalent to 30 $A_{260}^{cm^2}$.

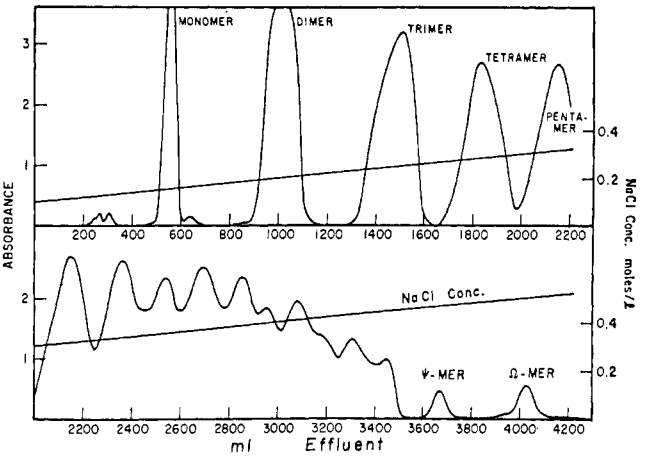

FIGURE 1: The chromatographic separation of oligonucleotides according to chain length. A column (1.5 × 60 cm) containing 100 ml of DEAE-Sephadex A-25 was loaded with 200 mg of a T_1 RNase hydrolysate of TMV RNA. The elution was performed with a linear concentration gradient from 0.1 to 0.6 M NaCl in 5 l. of 7 M urea (pH 7.5) at a flow rate of 40 ml/hr.

part of the elution procedure. If the gradient is begun immediately, Cp and ApCp will elute together. A linear gradient was run at a flow rate of 1.7 ml/hr from 0.001 M HCl to 0.026 M HCl–0.01 M KCl. The total volume of the gradient was 250 ml. At approximately the midpoint of the pH gradient, the point at which Up is eluted, a salt gradient was superimposed by adjusting the reservoir chamber of the gradient apparatus to a concentration of 0.1 M KCl by the addition of 2 M KCl.

Recovery of the Ω- and Ψ-mers following Chromatography. The fractions containing the long oligonucleotides were diluted fivefold with distilled water and passed through a DEAE-Sephadex column, 1.0 ml-bed volume (0.35 × 10 cm), at a flow rate of 50 ml/hr. The long T_1 oligomers remained bound on the column which was then washed with 5 ml of 7 M urea. The oligonucleotides were quantitatively eluted in a volume of 1–2 ml by passing a solution of 7 M urea containing 1.0 M NaCl through the column. Upon the addition of four volumes of ethanol to the column eluent, at 0°, the oligonucleotides precipitated while the NaCl and urea remained in solution (KCl will precipitate under these conditions). After standing for at least 4 hr at 0°, the precipitates were collected by centrifugation, washed with 95% ethanol, dried for 30 min under vacuum, and stored at −20°.

Sedimentation Equilibrium. Sedimentation equilibrium experiments on the Ω-mer were performed at 0° in 0.1 M NaCl–0.05 M $NaPO_4$–0.001 M NaEDTA buffer (pH 7.5). The rotor speed and column height were of the order of 10,000 rpm and 5 mm, respectively. The mean radius was 6.9 cm. Calculations of the molecular weight were based upon a solvent density of 1.010 g/ml and a partial specific volume for sodium RNA of 0.54 ml/g (Tennent and Vilbrandt, 1943).

Analysis of the 3'-Linked Terminus of the Ω-mer by ^{32}P Labeling. [γ-^{32}P]ATP was made by the method of Glynn and Chappell (1964). Polynucleotide kinase was generously provided by Dr. M. Takanami. Ω-mer (0.2 mg) was dissolved in 1.0 ml of buffer (0.1 M Tris–0.02 M $MgCl_2$–0.01 M mercaptoethanol, pH 7.4) containing 0.5 mole of [γ-^{32}P]ATP (approximately 10^7 cpm). This was mixed with 1.0 ml of polynucleotide kinase containing 50 units of enzyme (Takanami, 1967) and incubated at 37° for 1 hr. In a previous experiment, maximum incorporation was achieved after 0.5 hr under these conditions. At the end of the incubation, the mixture was freeze dried, taken up in 2 ml of 7 M urea, and applied to a 0.5 × 30 cm column of DEAE-Sephadex A-25.

The portion of the column eluent containing the ^{32}P-labeled Ω-mer was dialyzed and freeze dried. The labeled Ω-mer was next dissolved in 2 ml of 0.01 M Tris (pH 7.4) and to this was added 0.1 ml of a 0.2% (by weight) solution of pancreatic RNase. This mixture was incubated for 6 hr at 40°. At the end of this period, 0.1 mg of bentonite was added to the incubation mixture to absorb the nuclease (Singer and Fraenkel-Conrat, 1961). The mixture was centrifuged at 10,000 rpm to sediment the bentonite–nuclease complex and the supernatant was freeze dried. The dried material was dissolved in 2 ml of 7 M urea containing 1 mg of pancreatic RNase fragments of TMV RNA, to serve as chain-length markers, and was chromatographed on DEAE-Sephadex A-25. The portion of the column eluent containing the major part of the radioactivity was combined with approximately 0.1 mg each of ADP, CDP, GDP, and UDP. This mixture was diluted with four volumes of water and applied to a 0.5 × 30 cm column of AG-1X2, −400 mesh. All radioactive samples were counted in a scintillation counter as described by Takanami (1967).

Results

Isolation of Unique Oligomers. When a T_1 RNase hydrolysate of TMV RNA is chromatographed in a system designed to separate oligonucleotides on the basis of chain length, the elution profile shown in Figure 1 is obtained. The longest chain-length component of the elution profile has been designated as the Ω fraction and the penultimate peak as the Ψ fraction (Mandeles, 1968). The yields of oligonucleotide in the Ψ and Ω fractions are summarized in Table I. These yields were found to depend heavily upon the flow rate of the column. When the flow rate of the system described in Figure 1 was increased from 40 to 100 ml per hr, the yield of material in the Ω fraction dropped to half of the original

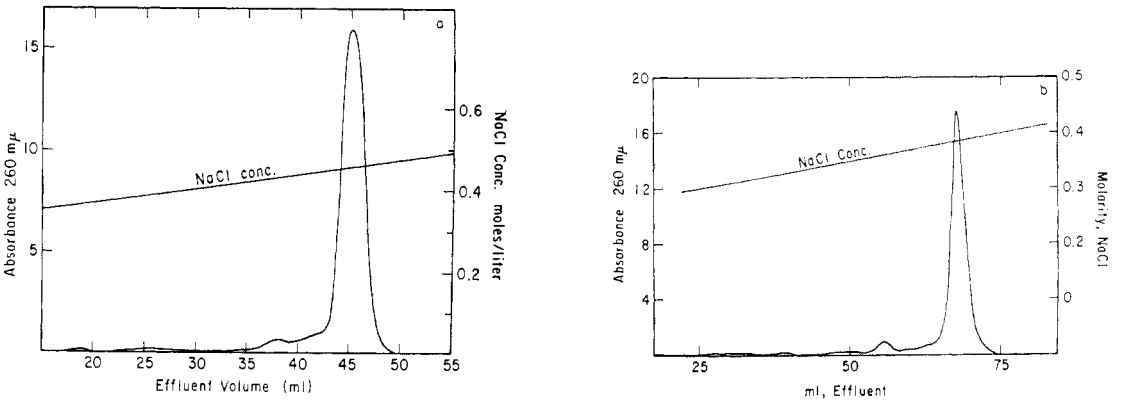

FIGURE 2: The rechromatography of (a) the Ψ-mers and (b) the Ω-mer. A column (0.4 × 25 cm) containing 3 ml of DEAE-Sephadex A-25, was loaded with 1.5–2.0 mg of Ψ-mers or Ω-mer. The elution was performed with a linear concentration gradient from 0.25 to 1.0 M NaCl in 250 ml of 7 M urea (pH 7.5) at a flow rate of 2 ml/hr.

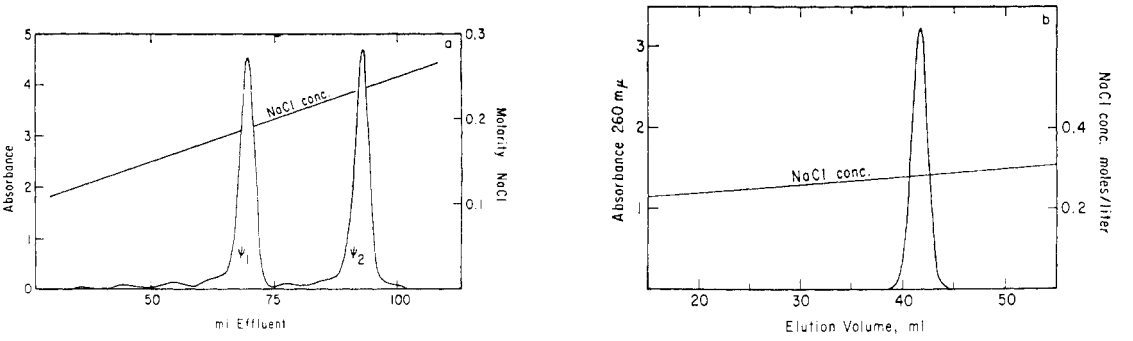

FIGURE 3: The low pH chromatography of (a) the Ψ-mers and (b) the Ω-mer. A column (0.4 × 35 cm) containing 4 ml of DEAE-Sephadex A-25 was loaded with the rechromatographed Ψ-mers or Ω-mer. The elution was performed with a linear concentration gradient from 0.05 to 0.55 M NaCl in 250 ml of 7 M urea (pH 2.8) at a flow rate of 1.8 ml/hr.

value. On the other hand, when the flow rate was decreased to 20 ml/hr, the yield of the Ω fraction was increased by only 5–10% an amount which is on the order of the experimental error. The dependence of the yield upon the flow rate can be attributed to the fact that at large flow rates the eluent does not have time to equilibrate with the adjacent resin particles. This departure from equilibrium causes the sample to be eluted in a broad band with long tails. Thus a smaller per cent of the sample is collected in the peak fractions. This effect will be most pronounced for long fragments, which, due to their lower diffusion coefficients, require a longer period of time to reach equilibrium.

Rechromatography of the Ψ and Ω fractions in the chain-length separation system depicted in Figure 2a,b did not yield any further resolution. However, since the rechromatography did appear to remove small amounts of impurities contained in the original Ψ and Ω fractions, this step was retained in the routine isolation of these oligonucleotide fractions. As shown in Figure 3a, the Ψ fraction was resolved into Ψ_1 and Ψ_2 fractions by further chromatography at pH 2.8. In contrast to the behavior of the Ψ fraction, the Ω fraction chromatographed as a single peak at pH 2.8. Figure 3b also indicates that the rechromatographed fraction was free of minor contaminants. For this reason the low pH chromatography was not routinely employed in the isolation procedure. The results obtained from the isolation procedure and oligonucleotide mapping studies (Mandeles, 1968) both suggest that each of the three oligonucleotide fractions is homogeneous and contains a nucleotide sequence unique to some location in the TMV RNA molecule.

An estimate of the chain length of the Ω-mer was made by sedimentation equilibrium. The values of the apparent molecular weight obtained at various times during the experiment are given in Table II. The value of 23,000 for the apparent molecular weight at 76 hr was taken as a good approximation to the true molecular weight. A value of 354 was calculated for the average molecular weight of a nucleotide plus its sodium counter ion. Since the Ω-mer was produced by exhaustive hydrolysis with T_1 RNase, and should therefore contain a single terminal guanine residue, the molecular weight of guanylic acid was not included in the average. From the above values a chain length of 65 nucleotides was calculated for the Ω-mer. Even allowing for extreme fluctuation in nucleotide composition, this value was estimated to differ from the true chain length by no more than plus or minus ten nucleotides.

Pancreatic RNase Hydrolysis. The Ψ-mers and Ω-mer were each digested with pancreatic RNase and the products were separated and identified. In some preliminary work, exhaustive hydrolysis conditions as used by previous workers

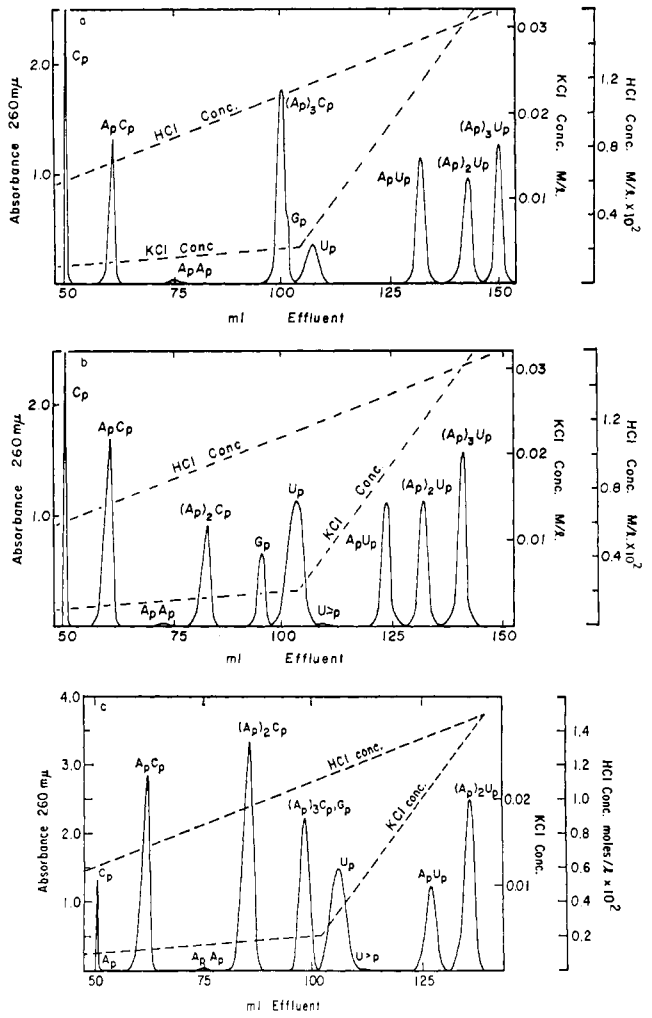

FIGURE 4: The separation of the pancreatic RNase digestion fragments of (a) the Ψ_1-mer, (b) the Ψ_2-mer, (c) the Ω-mer. A column (0.4 × 35 cm) containing 4 ml of DEAE-Sephadex, A-25 was loaded with 0.5 to 2 mg of a pancreatic RNase hydrolysate of the Ψ_1-mer, Ψ_2-mer, or Ω-mer. The elution was performed with a combination of gradients linear in KCl and HCl concentration.

(Rushizky and Knight, 1960) were employed to ensure complete hydrolysis. It was discovered that these conditions resulted in an appreciable amount of cleavage following adenosine. Beers (1960) has reported similar results. Our results indicate that the cleavage after adenosine does not occur randomly. For example, under the reaction conditions given in Table III, it was found that the hydrolysis of $(Ap)_3Cp$ with pancreatic RNase resulted in the production of four times more $(Ap)_2Cp$ than ApCp. These findings led to the adoption of the milder reaction conditions described earlier in this paper. These conditions were found to result in only 1–2% hydrolysis of $(Ap)_3Cp$ and leave less than 1% of the uridylic acid in the form of the cyclic diester.

TABLE II: Molecular Weight of the Ω-mer from Sedimentation Equilibrium.

Time (hr)	App Mol Wt (g/mole)
22	16,700
31	18,900
46	21,100
53	21,700
76	23,000

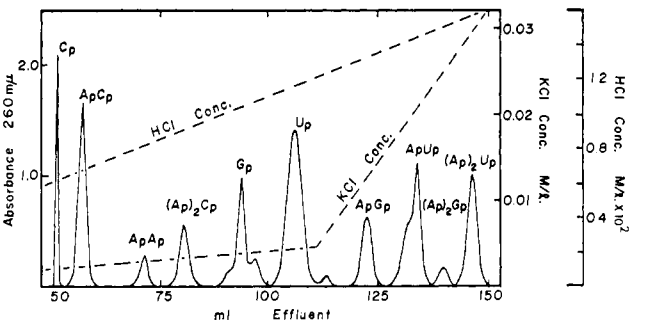

FIGURE 5: The Separation of the pancreatic RNase digestion fragments of mixed T_1 RNase oligomers. The procedure is the same as that employed in Figure 4.

The pancreatic RNase hydrolysates were separated chromatographically on a DEAE-Sephadex column using a combination of linear KCl and HCl gradients. The oligomers were identified from their elution positions and a knowledge of the specificities of T_1 and pancreatic RNase. These assignments were checked by comparing the ultraviolet absorption spectra of the oligomers eluted from the column with those of known oligomers and, where necessary, by rechromatography with known oligomers. In all cases, the results lead to an unambiguous identification. The profiles shown in Figure 4a–c show the elution positions of the fragments obtained by pancreatic RNase digestion of the Ψ_1-mer, Ψ_2-mer, and Ω-mer. This separation technique is actually more versatile than is indicated by the above figures. Figure 5 shows the elution pattern obtained from a pancreatic RNase digest of mixed T_1 RNase oligomers of TMV RNA. One sees that it is also possible to resolve oligonucleotides of the form $(Ap)_nGp$.

TABLE III: Cleavages Resulting in a Terminal Ap Observed with Pancreatic Ribonuclease.[a]

Initial Oligomer	Products	% Hydrolysis Obsd
ApCp ApUp	None	0
ApApCp ApApUp	Ap, ApCp Ap, ApUp	10–15
ApApApCp	Ap, ApApCp ApAp, ApCp	8–10 32–40

[a] Hydrolysis conditions: 2.5–7.5 mg of RNA/ml in 0.025 M PO_4 (pH 7.9), 1:10 enzyme:substrate ratio (w/w) 20-hr incubation at 37°.

TABLE IV: Pancreatic RNase Fingerprints of the Ω-mer and Ψ-mers.

Digestion Fragment	Ω-mer		Ψ_1-mer		Ψ_2-mer	
	μmoles of Fragment[a]/ μmole of Gp	Closest Integral Value	μmole of Fragment[a]/ μmole of Gp	Closest Integral Value	μmole of Fragment[a]/ μmole of Gp	Closest Integral Value
Gp	1.00	1	1.00	1	1.00	1
Cp	1.05	1	3.86	4	2.08	2
ApCp	6.08	6	1.94	2	2.02	2
$(Ap)_2Cp$	6.04	6	Not present	0	1.06	1
$(Ap)_3Cp$	1.96	2	0.92	1	Not present	0
Up	12.00	12	2.04	2	5.30	5
ApUp	2.96	3	1.90	2	2.00	2
$(Ap)_2Up$	3.96	4	0.98	1	1.05	1
$(Ap)_3Up$	Not present	0	0.93	1	1.04	1
Ap	0.10	0	0.04	0	0.05	0
ApAp	0.03	0	0.02	0	0.02	0
	$A_{35}U_{19}C_{15}G$		$A_{12}U_6C_7G$		$A_{11}U_9C_5G$	

[a] This figure is the average of the values obtained from two experiments.

The portions of the eluent containing the pancreatic RNase digestion products were collected, and their volumes were measured. The ultraviolet absorption spectrum of each fraction was measured and the number of 260-mμ absorbance units present in the fraction was computed. These values were converted into micromolar values with appropriate extinction coefficients (Cantor and Tinoco, 1965; Warshaw and Tinoco, 1965). The three oligomers being investigated should each contain a single, terminal guanine residue. Thus, the mole ratios of each oligomer isolated form the pancreatic RNase digest, relative to guanylic acid, should have integral values equal to the number of moles of each oligomer per mole of Ψ_1-mer, Ψ_2-mer, or Ω-mer. Table IV shows the mole ratios obtained for two different digests of each of the three T_1 RNase fragments. $(Ap)_3Cp$ and Gp were eluted as a single peak from the initial column and were separated by rechromatography at neutral pH as shown in Figure 6. The results of this rechromatography were corrected to 100% yield (from 95%) for comparison with the values obtained from the original column. The small amounts of uridine cyclic phosphate present were included in the uridine 3′-phosphate tally. The values given for ApAp were estimated to ±20% from the area under the elution profile. The Cp eluted from the column was contaminated with small amounts of Ap. The amount of Cp present was calculated from ultraviolet absorption spectra. Since the spectra of Cp and Ap are quite distinct, and since the amount of Ap present was small, this procedure gives very accurate values for Cp but rather poor values for Ap (about ±20%). Between 97 and 98% of the ultraviolet-absorbing material present in the pancreatic RNase digests is accounted for by the oligomers shown in Table IV. There is no evidence, either from the spectroscopic data or from rechromatography of the various oligomer fractions, which indicates the presence of modified bases in the hydrolysates. The results of the analyses of the pancreatic RNase products are shown in Table IV. It may be seen that the Ω-mer has a chain length of 70 nucleotides, which is in agreement with the sedimentation equilibrium measurement, and the Ψ-mers both have chain lengths of 26 nucleotides.

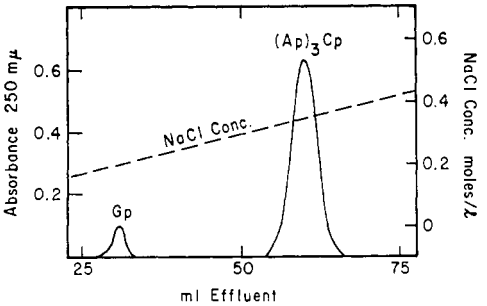

FIGURE 6: The rechromatography of the Gp, $(Ap)_3Cp$ peak from Figure 4c. A column (0.35 × 10 cm) containing 1 ml of DEAE-Sephadex was loaded with the Gp, $(Ap)_3Cp$ peak from Figure 4c. The elution was performed with a linear gradient from 0 to 1.5 M NaCl (pH 7.0) in 250 ml at a flow rate of 1.2 ml/hr.

End-Group Analyses. In addition to the determination of the pancreatic RNase fingerprints, the terminal nucleotides at both ends of the Ω-mer have been identified. The nucleotide at the 3′-linked terminus of the Ω-mer was found to be uridylic acid. This determination was made in the following manner. The 5′-hydroxyl group at the 3′-linked terminus of the Ω-mer was esterified with ^{32}P according to

$$*pppA + HO\text{-}Xp\text{-}(Xp)_n \xrightarrow{\text{polynucleotide kinase}} ppA + *pXp\text{-}(Xp)_n$$

This reaction is catalyzed by the enzyme polynucleotide kinase isolated from T_4 bacteriophage infected *Escherichia coli* (Richardson, 1965; Novogrodsky and Hurwitz, 1965).

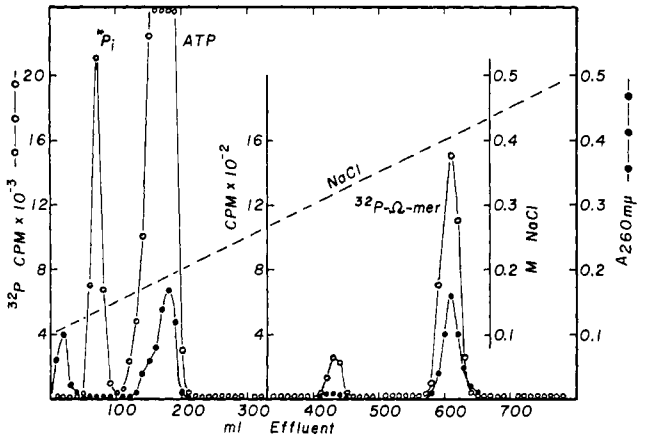

FIGURE 7: The isolation of [5′-^{32}P]Ω-mer. A column (0.5 × 30 cm) containing 6 ml of DEAE-Sephadex A-25 was loaded with a polynucleotide kinase reaction mixture containing approximately 0.2 mg of [5′-^{32}P]Ω-mer. The elution was performed with a linear concentration gradient from 0.1 to 0.6 M NaCl in 1000 ml of 7 M urea (pH 7.5).

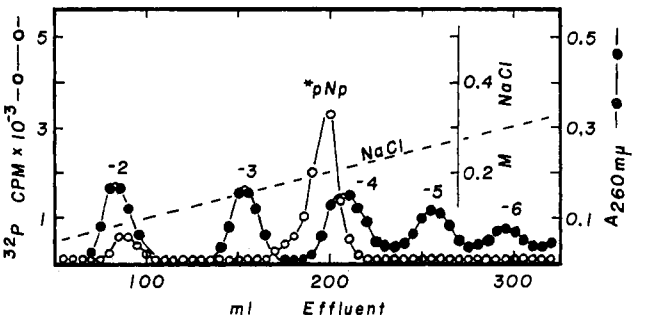

FIGURE 8: The chain-length separation of the pancreatic RNase fragments of [5′-^{32}P]Ω-mer. A column (0.5 × 30 cm) containing 6 ml of DEAE-Sephadex, A-25 was loaded with a pancreatic RNase digest of [5′-^{32}P]Ω-mer and 1 mg of pancreatic RNase fragments of TMV RNA as chain-length markers. The elution was performed with a linear concentration gradient from 0 to 0.5 M NaCl in 500 ml of 7 M urea (pH 7.5).

Figure 7 shows the chain-length separation of the polynucleotide kinase reaction products. The first peak of radioactive material is eluted in a position corresponding to mononucleotides with two negative charges and represents inorganic orthophosphate which is probably produced through hydrolysis of a small amount of [^{32}P]ATP. The second peak of radioactive material is unreacted [^{32}P]ATP. The last peak in the elution profile occurs in the position expected for the Ω-mer. The labeled Ω-mer was hydrolyzed with pancreatic RNase and the digestion products were chromatographically separated according to their chain lengths. The elution profile which was obtained is shown in Figure 8. The major portion of the radioactivity was eluted in the position expected for a nucleoside diphosphate which possesses four negative charges at neutral pH. When the labeled nucleoside 3′,5′-diphosphate was rechromatographed at low pH with nucleoside 5′-diphosphate markers, as shown in Figure 9, it was eluted in a position corresponding to *pUp (Takanami, 1967). In order to be certain that a significant portion of the Ω-mer molecules was actually being labeled, the per cent of phosphate incorporation was calculated. The specific activity of the ATP was 13.2 × 10^6 cpm/μmole. The specific activity of the labeled Ω-mer was measured as 18.6 × 10^3 cpm/$A_{260}^{cm^2}$, from which one calculates that 1.4 × 10^{-3} μmole of phosphate was incorporated per $A_{260}^{cm^2}$ of Ω-mer. The data from the fingerprint experiments indicate an average residue molar extinction coefficient of 10.5 × 10^3 for the Ω-mer. Since the chain length of the Ω-mer is 70, one calculates the per cent of molecules labeled with phosphate to be 104%. This shows that uridine is the nucleoside located at the 3′-linked terminus of the Ω-mer.

We have obtained a tentative sequence for the 5′-linked end of the Ω-mer. An acid-soluble nuclease which occurs as an impurity in commercial alkaline phosphatase (Worthington BAP-SF) has been described by Anderson and Carter (1965). They have shown that this nuclease possesses some specificity for phosphodiester linkages following cytidine. The nuclease was prepared and assayed as described, and in a single preliminary experiment, a 0.5-mg sample of Ω-mer was hydrolyzed with 5 units of this acid-soluble nuclease in 0.3 ml of 0.04 M Tris-Cl buffer (pH 8.0). The reaction was carried out at 37° for 20 hr. The hydrolysis fragments were separated in the paper electrophoresis–chromatography system of Rushizky and Sober (1962). The isolated fragments were eluted from the paper and hydrolyzed with pancreatic RNase. Only one of the initially isolated fragments was found to contain guanylic acid. The pancreatic RNase digestion products of this fragment were identified from their chromatographic behavior as $(Ap)_2Up$ and Gp. Thus, the terminal sequence, CpApApUpGp, has been tentatively assigned to the Ω-mer.

Discussion

It is not known what proteins, if any, are produced by the portions of TMV RNA which contain the Ω-mer and Ψ-mers. However, none of these fragments can be a part of the gene for the coat protein. Knowledge of the genetic code (Nirenberg *et al.*, 1966) and the amino acid sequence of TMV coat protein (Funatsu *et al.*, 1964) indicates that the coat protein gene cannot give rise to T_1 RNase fragments of chain lengths

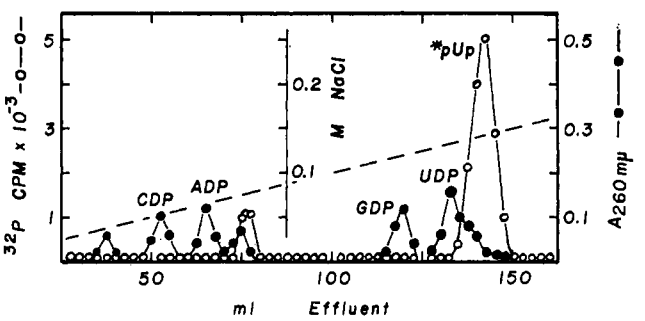

FIGURE 9: The isolation of the labeled terminus of the Ω-mer. A column (0.5 × 30 cm) containing 6 ml of AG1×2 (−400 mesh) was loaded with the labeled fraction from Figure 8 and with 0.1-mg quantities of ADP, CDP, GDP, and UDP as markers. The elution was performed with a linear concentration gradient from 0 to 0.5 M NaCl in 500 ml of dilute HCl (pH 2.0).

26 or 70. This observation is in agreement with the findings of Kado and Knight (1966, 1968) who have determined that both the local lesion gene and the coat protein gene are located in the 3′-linked-half of the RNA strand. The Ψ-mers and Ω-mer are all located nearer to the 5′-linked end of the TMV RNA molecule (Mandeles, 1968).

The Ω-mer stands out among the three fragments which have been investigated because of its base composition: it possesses a sequence of 69 nucleotides from which Gp is totally absent. and roughly half of the 69 nucleotides are Ap. As for the Ψ-mers, it appears that these two fragments may possess complete homology except for three nucleotide changes. This can be seen if the pancreatic RNase fragments from the two Ψ-mers are written as follows:

Ψ-1: C, C, AAAC, C, C, AC, AC, U, U, AU, AU, AAU, AAAU, G

Ψ-2: U, U, U, AAC, C, C, AC, AC, U, U, AU, AU, AAU, AAAU, G.

It has been suggested (Cantor and Jukes, 1966) that one might expect to find homologies between different genes in the same cell as a result of gene doubling during the evolutionary process. The question remains whether the similarity observed between the two sets of pancreatic RNase fragments is merely coincidence or whether the similarity is unusual enough to suggest that the apparent homology actually exists. The results of a combinatorial calculation based on a random distribution of nucleotides in TMV RNA indicate that the odds of obtaining the observed similarities for the Ψ-mers are about 1 in 25,000.

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