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STRUCTURAL DIFFERENCES IN THE NUCLEIC ACIDS OF SOME TOBACCO MOSAIC VIRUS STRAINS*

I. MONOPYRIMIDINE NUCLEOTIDES IN RIBONUCLEASE DIGESTS

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Attempts to correlate the distinctive biological differences exhibited by the tobacco mosaic virus (TMV) strains with the chemical composition of their protein and nucleic acid components have been made¹. The purine and pyrimidine composition of their nucleic acid part^{2,3} and that of ribonuclease resistant residues (precipitable by 6% trichloroacetic acid) obtained after exhaustive digestion of each of the nucleic acids⁴, have been found to be similar. If the nucleic acids represent the genetic material of viruses^{5,6}, it would seem that the nucleic acids of viral strains must differ in at least some structural features. Although there is no difference in the base composition of the strain nucleic acids and their ribonuclease resistant residues, it is probable that the way the individual nucleotides are arranged in each case might be different. Hence it was thought an examination of ribonuclease digests might reveal some differences in the intramolecular distribution of pyrimidine nucleotides in the nucleic acids of TMV strains.

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The present communication deals with the quantitative estimation of monopyrimidine nucleotides, uridylic acid and cytidylic acid, present in the ribonuclease digests of each of the nucleic acids of four strains of TMV. The monopyrimidine nucleotides were found to be the same in amount in the digests of the nucleic acids of the strains, TMV, HR and YA, while in the digest of M strain nucleic acid these were present in significantly larger amounts. This appears to be the first evidence of the structural differences in the nucleic acids of different strains of a virus.

MATERIALS AND METHODS

Nucleic acids were isolated from the purified preparations of each virus according to the method of COHEN AND STANLEY⁷ as modified by KNIGHT⁸.

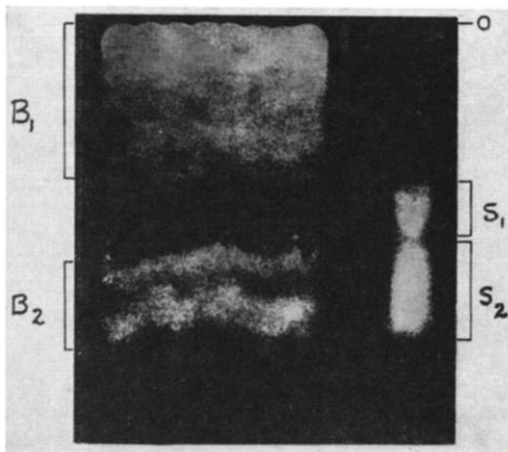
Pancreatic ribonuclease used in these studies was a crystalline preparation obtained from the Worthington Corporation, Freehold, New Jersey.

Rate of appearance of pyrimidine mononucleotides in the ribonuclease digests of TMV nucleic acid

In order to establish conditions for the treating of nucleic acids of different strains with ribonuclease a preliminary experiment was done with TMV nucleic acid. Five mg of nucleic acid were dissolved in 0.5 ml of 0.02 *M* phosphate buffer at pH 7.6. To this was added 125 γ of ribonuclease dissolved in 0.5 ml of 0.02 *M* phosphate buffer at pH 7.6, and the mixture was incubated at room temperature (23°). At known intervals of time (Fig. 2) 0.18 ml aliquots were withdrawn, placed on a Whatman 3 mm filter paper (55 × 24 cm) in a band at a distance of 10 cm from the top of the filter paper, and dried in a current of air. On the same paper a mixture of cytidylic acid, uridylic acid, guanylic acid and adenylic acid was run side by side. The chromatogram was developed in *isopropanol*-water-ammonia solvent system⁸ for 24 hours at room temperature. The developed chromatogram was dried at room temperature and Fig. 1 is a photograph of the chromatogram of 12-hour ribonuclease digest taken according to the modified technique of SMITH AND ALLEN⁹. Band 2 in Fig. 1 corresponds to the reference spot containing adenylic acid, cytidylic acid and uridylic acid. Band 2 was eluted with distilled water and the eluate was concentrated to dryness in a vacuum desiccator over anhydrous CaCl₂. The residue was taken up in 0.1 ml of 0.01 *N* HCl. An aliquot was placed on a Whatman 3 mm filter paper and the chromatogram was developed in *isopropanol*-HCl-water solvent system¹⁰ for 18 hours at room temperature. After drying the chromatogram at room temperature, it was viewed under ultraviolet light. Only two spots corresponding to cytidylic acid and uridylic acid were noticed. These were marked with a pencil, cut out and eluted with 0.01 *N* HCl. Their amounts were determined spectrophotometrically.

From this preliminary experiment a digestion period of 12 hours was chosen for the reaction of nucleic acids of different strains with ribonuclease.

Fig. 1. Chromatogram of 12-hour ribonuclease digest of TMV nucleic acid at 23° and pH 7.6, developed in *isopropanol* (700 ml), water (300 ml) and 0.35 ml NH₃ solution, per litre of air space for 24 hours at 23°. O, origin of chromatogram; Band 1, a mixture of di-, tri- and tetra- etc. nucleotides; Band 2, monopyrimidine nucleotides; S₁, reference substance, guanylic acid; S₂, a mixture of reference substances, adenylic acid, cytidylic acid and uridylic acid.



Digestion of nucleic acids of different strains with ribonuclease

Two mg of the nucleic acids of four different strains of TMV^{2,11,12} were dissolved in 0.2 ml of 0.02 *M* phosphate buffer at pH 7.6. To each was added 50 γ of ribonuclease dissolved in 0.2 ml of 0.02 *M* phosphate buffer at pH 7.6 and the mixtures were incubated at room temperature (23°).

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for 12 hours. At the end of this period 0.2 ml of each of the digests was withdrawn and chromatographed. Cytidylic acid and uridylic acid were separated and estimated as described above. The results are given in Table I.

RESULTS AND DISCUSSION

In the ribonuclease digests of TMV nucleic acid, only the monopyrimidine nucleotides, uridylic acid and cytidylic acid, were observed and this is in accordance with the earlier observations with the yeast ribonucleic acid^{13,14}. There was a gradual increase in the appearance of cytidylic acid and uridylic acid in the digest with time and this attained a maximum by 12 hours and remained so after digestion for 18 hours (Fig. 2). In 12-hour and 18-hour digests, about 50% of uridylic acid and 46% of cytidylic acid present in the intact TMV nucleic acid were found.

The amounts of monopyrimidine nucleotides in the 12-hour ribonuclease digests of TMV, HR and YA were the same, while in the digest of M-strain nucleic acid there was a significant difference (Table I). Since the base composition of all the strain nucleic acids (TMV, HR, YA and M) is the same^{2,3}, the greater amount of monopyrimidine nucleotides in the ribonuclease digest of M-strain nucleic acid can be attributed only to the difference in the arrangement of the pyrimidine nucleotides. Since pancreatic ribonuclease is a highly specific phosphodiesterase, which hydrolyses only secondary phosphate esters of pyrimidine ribonucleoside 3'-phosphates^{15,8,16}, the appearance of monopyrimidine nucleotides in the ribonuclease digests indicates that two or more pyrimidine nucleotides were linked together in the nucleic acid chains without intervening purine nucleotides (Fig. 3). The appearance of larger amounts of monopyrimidine nucleotides in the ribonuclease digest of M-strain nucleic acid is a clear indication of the occurrence of a larger proportion of pyrimidine polynucleotide segments than in the nucleic acids of TMV, HR and YA.

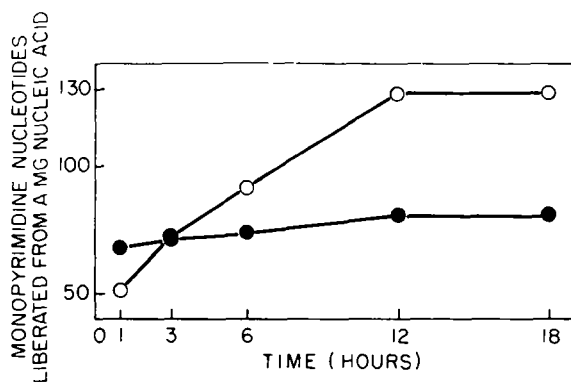


Fig. 2. Time course of liberation of monopyrimidine nucleotides, uridylic acid and cytidylic acid in the 12-hour ribonuclease digest of TMV nucleic acid. Reaction mixture consisted of 5 mg of TMV nucleic acid in 0.5 ml 0.02 *M* phosphate buffer at pH 7.6 and 125 γ of pancreatic ribonuclease in 0.5 ml 0.02 *M* phosphate buffer at pH 7.6, incubated at 23°. ○—○ uridylic acid; ●—● cytidylic acid.

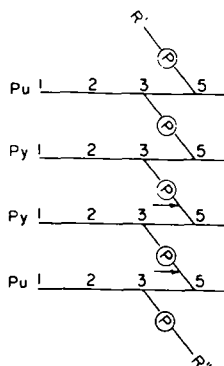


Fig. 3. Diagram of polyribonucleotide chain. P = phosphate group; pu = purine; py = pyrimidine; *R'* and *R''* = rest of the chain; → indicates the point of cleavage by ribonuclease

TABLE I
MONOPYRIMIDINE NUCLEOTIDES, URIDYLIC ACID AND CYTIDYLIC ACID IN THE TWELVE HOUR
RIBONUCLEASE DIGESTS OF TMV STRAIN NUCLEIC ACIDS

Strain	Preparation No.	UA			CA		
		γ-mg NA	Average	% of total UA in NA	γ-mg NA	Average	% of total CA in NA
TMV	1	126.2	127.5	50.7	80.8	79.9	45.7
	2	128.8			79.0		
HR	1	124.9	120.8	48.1	71.1	73.5	42.0
	2	116.6			76.0		
YA	1	128.2	124.0	49.3	76.3	78.8	45.0
	2	119.8			81.2		
M	1	185.4	178.1	70.8	107.7	107.1	61.2
	2	172.6			100.9		
	3	176.4			112.8		

UA = uridylic acid; CA = cytidylic acid; NA = nucleic acid.

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SUMMARY

The nucleic acids obtained from four strains of tobacco mosaic virus were digested with crystalline pancreatic ribonuclease at 23° for 12 hours at pH 7.6. The monopyrimidine nucleotides, uridylic acid and cytidylic acid, were isolated from such digests and estimated. Significantly greater quantities of pyrimidine mononucleotides were released from the nucleic acid of M strain than from those of strains, TMV, HR and YA. From this result it was concluded that the intramolecular distribution of pyrimidine nucleotides in the nucleic acid of M strain differs from those of strains, TMV, HR and YA.

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