ULTRAVIOLET ABSORPTION STUDIES ON TOBACCO MOSAIC VIRUS*

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The ultraviolet absorption of tobacco mosaic virus (TMV) is caused by the tyrosine, tryptophan and phenylalanine of protein and the purines and pyrimidines of nucleic acid. Very careful work on the ultraviolet absorption of TMV was carried out by BUTENANDT, FRIEDRICH-FREKSA, HARTWIG AND SCHEIBE[1]. However, during the purification of TMV the aggregation of virus particles takes place and this intensifies light scattering. This was not taken into consideration by BUTENANDT *et al.*[1]. Since the scattering increases with the decreasing wavelength its effect will be more pronounced in the ultraviolet region and thus contributes to an apparent increase in the absorption. SCHRAMM AND DANNENBERG[2] measured the spectrum of TMV, correcting for scattering. The exact amounts of absorption contributed by protein and nucleic acid components of TMV are not yet known. Such studies can tell something about the nature of the protein and nucleic acid binding in TMV. The present investigation deals with (1) the measurement of absorption spectrum of TMV after correcting for scattering, (2) the determination of absorption contributed by the protein part of TMV and (3) the comparison of the resulting absorption spectrum, obtained after deduction for the absorption contributed by the protein part of TMV, with that of TMV nucleic acid prepared using two different procedures.

EXPERIMENTAL

Materials

Tobacco mosaic virus was prepared by differential centrifugation and the final preparation was in aqueous solution. Nucleic acid from TMV was prepared by using two different procedures: (1) the heat denaturation method of COHEN AND STANLEY[3] as modified by KNIGHT[4] and (2) the detergent method of FRAENKEL-CONRAT, SINGER AND WILLIAMS[5]. Native TMV protein was prepared according to the procedure of FRAENKEL-CONRAT AND SINGER[6] with the following slight modification.

One per cent solution of TMV (250 mg) was dialysed against 1 litre of 1 % solution of 2-methyl-2-amino-1-propanol at 3° C for 24 hours (suggested by Dr. P. NEWMARK). Saturated $(NH_4)_2SO_4$ solution was added to the dialysed material to 10% saturation, held at room temperature for 1 hour and then centrifuged at 12,000 r.p.m. for 10 minutes. Concentration of $(NH_4)_2SO_4$ in the supernatant was raised to 30%, held at room temperature for one hour and then centrifuged at 12,000 r.p.m. for 10 minutes. The precipitate was dissolved in 7 ml distilled water and to this a saturated $(NH_4)_2SO_4$ solution was added to 30% saturation. After holding at room temperature for 1 hour, it was centrifuged for 10 minutes at 12,000 r.p.m. The precipitate was dissolved in 7 ml distilled water and dialysed at 3° C against two changes of 4 litres of distilled water

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adjusted to pH 7.5. The dialysed material was centrifuged for 2 hours at 40,000 r.p.m. The supernatant was used in the following studies.

Methods

Phosphorus was estimated according to the procedure of KING[7] with a slight modification in the digestion. The samples were digested for 15 minutes (in the case of TMV a digestion period of 30 minutes was necessary) at 180° C. The samples were cooled and digested for another 5 minutes following the addition of a drop of H_2O_2. Protein was estimated according to the method of HILLER, MCINTOSH AND VAN SLYKE[8]. The intensity of the colour was measured at 550 mμ in a Beckman spectrophotometer.

The absorption spectra of TMV and TMV nucleic acid were measured in 1 cm quartz cells in a Beckman spectrophotometer DU model.

Absorption spectra of TMV. The absorption of a TMV preparation containing 13 μg P in 10 ml distilled water was measured. The results, expressed as ε(P)[9] are given in Fig. 1, Curve I.

Scattering was measured using the Raleigh rule: $K = C/\lambda^4$ where K is extinction due to scattering, C is scattering constant, λ is wavelength in mμ. The exponent in the formula was verified by the author using the method of SCHRAMM AND DANNENBERG[2] and was found to be 4.0. The absorption of a TMV preparation containing 156 μg P in 10 ml distilled water was measured at wavelengths 350 and 400 mμ, where nucleic acid and protein have no absorption and the scattering constant C was calculated using the Raleigh formula. The constant C was found to be $5.34 \cdot 10^{-19}$ cm^3 (Table I). For the amount of TMV containing 13 μg P in 10 ml distilled water, which was used to obtain the spectrum of TMV (Fig. 1, Curve I), the scattering constant is $5.34 \cdot 10^{-19}/12$ or $0.45 \cdot 10^{-19}$ cm^3. This figure was substituted in the Raleigh formula to obtain values for scattering (K) at wavelengths 240 to 310 mμ, and Curve II in Fig. 1 was obtained after subtracting values due to scattering.

The absorption of 2.79 mg of native TMV protein in 10 ml distilled water was measured at wavelengths 240 to 310 mμ, and Curve III in Fig. 1 was obtained after deducting the absorption due to protein from Curve II in Fig. 1.

Absorption spectra of TMV nucleic acid. About 2 mg of nucleic acid were dissolved in 1 ml distilled water. Phosphorus was determined in an aliquot of this solution. Absorption measurements were made on 0.1 ml of this solution diluted to 10 ml with distilled water. The values, expressed as ε(P), are given in Fig. 2 a, b.

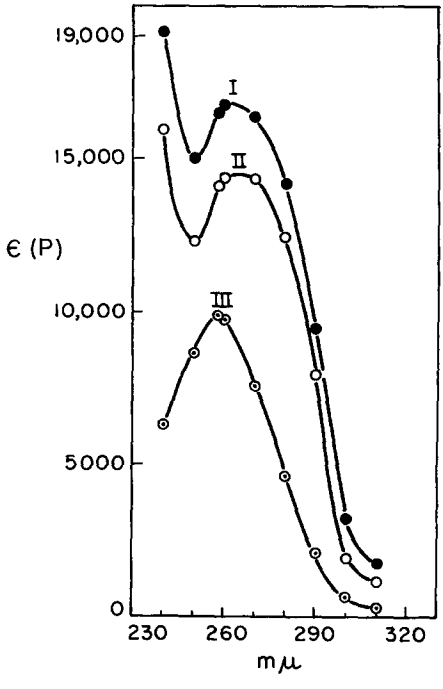

Fig. 1. Ultraviolet absorption of tobacco mosaic virus in distilled water. I. Ultraviolet absorption spectrum before deducting for scattering; II. Ultraviolet absorption spectrum after deducting for scattering; III. Ultraviolet absorption spectrum after deducting for scattering and protein.

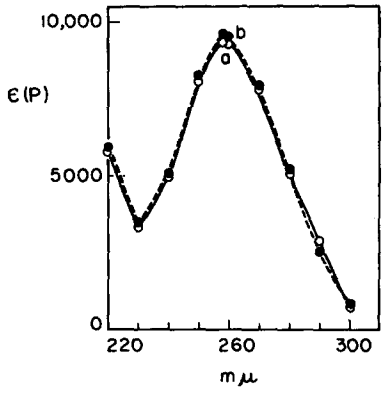

Fig. 2. Ultraviolet absorption spectra of TMV nucleic acid in distilled water. (a) Spectrum of TMV nucleic acid prepared according to heat denaturation method. (b) Spectrum of TMV nucleic acid prepared according to detergent procedure.

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TABLE I

SCATTERING CONSTANT C FOR TMV CONTAINING 156 μg P IN 10 ml DISTILLED WATER
$C = K \times \lambda^4$

Wavelength (λ) $m\mu$	$\log I/I_0$ (K)	$C \times 10^{19}$ cm^3
350	0.360	5.40
360	0.317	5.33
370	0.286	5.36
380	0.255	5.32
390	0.230	5.32
400	0.208	5.33
		Average 5.34

RESULTS AND DISCUSSION

The ultraviolet absorption spectrum of TMV in Fig. 1, Curve I, is contributed by (1) scattering, (2) protein and (3) nucleic acid. Curve II in Fig. 1, obtained after deducting values due to scattering, is composed of the contributions from both the constituents of TMV, protein and nucleic acid with ε(P) values, 12,290 and 14,400 at 250 mμ and 260 mμ respectively. Curve III in Fig. 1, obtained after subtracting absorption contributed by protein from Curve II, represents the absorption due to the nucleic acid part of TMV. The two TMV nucleic acid preparations, made according to heat denaturation and detergent procedures (Fig. 2a, b), and Curve III in Fig. 1, have an absorption maximum at 258 mμ with ε(P) values of 9350, 9674 and 9845 respectively. It is interesting to note that there is no significant difference in the ε(P) values. If the purines and the pyrimidines of TMV nucleic acid are involved in the formation of the nucleoprotein complex (TMV) in a way to suppress some of the chromophores, one expects the resultant Curve III, obtained after deducting the absorption values of the protein part from the TMV absorption curve, to have a low absorption maximum. Since this is not the case it is probable that the purines and pyrimidines do not participate in the formation of the nucleoprotein complex (TMV). It is likely that the main linkage between the protein and nucleic acid in TMV is that involving the phosphorus groups of nucleic acid with the guanidino groups of the basic amino acid, arginine, of TMV protein.

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SUMMARY

The ultraviolet absorption of tobacco mosaic virus was studied. After correcting for the absorption contributed by the protein part of TMV and scattering, the resulting curve has about the same absorption maximum as that found for TMV nucleic acid prepared using two different procedures. The significance of this result on the nature of the protein-nucleic acid association in TMV has been discussed.

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KINETIC STUDIES ON THE DIPHOSPHOPYRIDINE NUCLEOTIDE CYTOCHROME *c* REDUCTASE FROM HEART*

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A soluble, highly purified DPNH**-cytochrome *c* reductase has been obtained by MAHLER *et al.*[1] from heart muscle sarcosomes. The overall stoichiometry of the reaction catalyzed by the flavoprotein was found to be:

$$\text{DPNH} + 2\ \text{cytochrome}^{3+} \rightleftharpoons \text{DPN}^{+} + 2\ \text{cytochrome}^{2+} + \text{H}^{+} \qquad (1)$$

The reaction therefore involves the participation of three substrate molecules, two of which are proteins. Numerous problems are posed by this reaction, some of which may be approached by kinetic analysis. A preliminary survey of the kinetics has been reported[2]. The kinetics of the overall reaction are here subjected to a more extended experimental analysis. The objects were to determine the values of the kinetic parameters involved in the reaction, to discuss their nature, and to determine their behavior as a function of pH. The results so obtained form the basis for a discussion of the possible mechanism of the reaction.

MATERIALS AND METHODS

Preparation of the enzyme

The enzyme was prepared from a dilute alcohol extract of pig heart sarcosomes by a slight modification of method of MAHLER *et al.*[1]. Removal of the contaminating heme pigments was facilitated by washing the sarcosomes with cold 0.05 *M* sodium acetate buffer at pH 5.4 and then several times with cold distilled water. The optical density ratios D_{280}/D_{440} and D_{410}/D_{440} of the enzyme at the last stage of purification were 5.1 and 0.95. The values of these ratios obtained by MAHLER *et al.*[1] were 7.0 and 0.85. Although electrophoretic and sedimentation studies indicated that the

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** The following abbreviations are used: DPN^{+} and DPNH, unreduced and reduced forms of diphosphopyridine nucleotide, respectively; tris, tris(hydroxymethyl)aminomethane; cyt, cytochrome *c*.

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