Conformational Studies on Particles of Turnip Yellow Mosaic Virus

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SUMMARY

Circular dichroism studies (CD) of turnip yellow mosaic virus (TYMV) nucleoprotein and of its isolated RNA and capsid revealed that: (i) the nucleic acid structure, which comprises a considerable amount of base pairing and/or stacking, remains essentially unchanged irrespective of whether the RNA is encapsidated or free; (ii) the secondary structure of the protein component is mainly accounted for by β- and irregular forms without appreciable amounts of α-helix; (iii) the interaction of capsid protein and RNA induces some conformational changes in the protein probably involving a decrease of β-structure and a perturbation of the microenvironment of some aromatic residues. The influence of temperature on the CD spectra of virus nucleoprotein, RNA and capsid was also investigated. The results are discussed in connection with particle stability.

INTRODUCTION

Turnip yellow mosaic virus (TYMV) is one of the most extensively studied isometric plant viruses. The fundamental work of Kaper and co-workers has produced a considerable insight into the protein–protein, protein–RNA and RNA–RNA interactions contributing to the stabilization of the nucleoprotein particle (Kaper, 1975).

X-ray diffraction and electron microscope studies (Klug et al. 1966; Finch & Klug, 1966) showed that the single-stranded RNA penetrated the protein shell deeply at high ionic strength, somewhat following the icosahedral symmetry of the protein subunit arrangement. However, a recent neutron small-angle scattering study (Jacrot et al. 1977) indicated little, if any, penetration of the RNA into the protein capsid at low ionic strength. In any case, until recently, detailed information was missing about the conformation of protein and RNA components, either when integrated in the virion or when in an isolated state. The only data available are those obtained with Laser–Raman spectroscopy of whole virus by Turano et al. (1976).

In an attempt to obtain a clearer insight into the conformation of TYMV virions, investigations by circular dichroism (CD) were made on intact virus particles and on their isolated moieties. The effects of temperature and of wide changes in ionic strength were also studied in view of the well established dependence of TYMV stabilizing interactions on these factors (Lyttleton & Matthews, 1958; Kaper, 1971; Piazzolla et al. 1977a). Therefore, this study complements and extends the investigations of Turano et al. (1976).
Fig. 1. CD spectra at room temperature of TYMV nucleoprotein (---) and TYMV-RNA (----).

Fig. 2. Fractional ellipticity of TYMV protein component observed for capsids (---) or calculated by subtraction of values for the free RNA from those for the virus nucleoprotein (----).

METHODS

Virus purification and fractionation. The virus used in these studies was a TYMV isolate kindly supplied by Dr J. M. Kaper. It was propagated in Chinese cabbage grown in a controlled environment glasshouse and purified according to Dunn & Hitchborn (1965). TYMV capsids (T component) and TYMV-RNA were prepared by heating virus preparations at 76 °C for 90 s in 0.02 M-K-Na phosphate buffer, pH 7.2, containing 1 M-NaCl (Piazzolla et al. 1977a). After cooling quickly to 0 °C, the dissociated virus was diluted 10-fold with 0.02 M-phosphate buffer, pH 7.2, and fractionated by sucrose (0.2 to 0.8 M) density gradient centrifugation in a Beckman SW 25.1 rotor at 24,000 rev/min for 4 h (T component) or 14 h (RNA).

CD measurements. CD spectra were obtained with a Cary 61 dichrograph, using the same technique and suspending medium [0.02 M-K–Na phosphate buffer, pH 7.2, plus 0.1 M-NaCl (phosphate buffer), unless otherwise stated] as previously reported (Piazzolla et al. 1977b). The data are expressed in terms of either [θ], the mean (nucleotide or amino acid) residue molecular ellipticity in units of degrees cm²/dmol or χ(θ), the partial specific ellipticity in units of degrees cm²/dg, where [θ] is the specific ellipticity and χ is the weight fraction of the respective component in the virus. Calculated mean residue mol. wt. were 335.7 for RNA and virus nucleoprotein and 106.5 for the capsid.

Analytical determinations. Molar base composition of TYMV-RNA is known to be: A, 22.4%; U, 22.1%; G, 17.2%; and C, 38.3%, the weight fraction of RNA being 0.334 (Kaper, 1975). The concentrations were determined spectrophotometrically using the following absorption coefficients: A 260nm = 8.6, A 270nm = 1.1 and A 280nm = 25 for virus nucleoprotein, T component and RNA respectively (Kaper & Alting Siberg, 1969).
RESULTS

CD spectra of virus and its components

The CD spectra of TYMV and of its RNA are shown in Fig. 1. In the longer wavelength region, where the dichroic absorption is essentially due to the nucleic acid, the spectra are very similar, suggesting structural analogies between isolated and intraviral RNA. According to current interpretations (Yang & Samejima, 1969; Gratzer & Richards, 1971; Piazzolla et al., 1977b), the spectra indicate the presence of a significant amount of base pairing and stacking in the single-stranded RNA. A maximum of 55 to 60 % was found by Turano et al. (1976) by Laser–Raman spectroscopy.

In Fig. 2 the CD spectrum of artificial T component is reported (natural T component showed essentially the same spectrum). In the aromatic spectral range, one maximum at 293 nm and two minima at 280 and 240 nm are observed. In addition, a 255 nm peak and a 285 nm shoulder are present. In the far ultraviolet range the curve is characterized by a minimum at about 213 nm and by a maximum at 192 to 193 nm.

On the basis of 33 % RNA in the virus nucleoprotein, the RNA contribution can be subtracted from the curve of the nucleoprotein, assuming that RNA conformation is substantially unchanged after extraction. Such a calculated curve is also shown in Fig. 2. The presence of the nucleic acid enhances the protein optical activity in the aromatic region (except in the case of the small positive band at 293 nm) and decreases it in the amide absorption region. Apparently, RNA induces a conformational change in the protein component by decreasing the amount of the orderly secondary structure and also producing changes in the tertiary structure as reflected by the increase of the optical activity of some aromatic residues.
Temperature studies

The CD changes at selected temperatures of virus nucleoprotein and isolated RNA are shown in Fig. 3 and 4. Heating particularly affects the positive band of longer wavelength which shows a temperature dependence that is very similar for virus nucleoprotein and RNA. This again suggests remarkable similarity in the structure of isolated and intraviral RNA. This is made evident in Fig. 5 which shows dichroic absorbance profiles for isolated RNA and virus nucleoprotein at two ionic strengths, 0.1 and 1 M NaCl. Under these conditions, at pH near neutrality, virus dissociates between 45 and 65 °C but, whereas integrity of the capsid is maintained at higher ionic strength, at lower ionic strength some structural damage is produced (Lyttleton & Matthews, 1958; Kaper, 1971; Piazzolla et al. 1977a).

As far as virus nucleoprotein is concerned, Fig. 5 shows that, on increasing ionic strength, heat induced transition of RNA begins at higher temperature, a small amount of change being observed as a consequence of a large reduction in ellipticity at low temperature. To obtain information about the possible cooperativeness of the nucleic acid melting process, the data were analysed according to the van't Hoff equation (Brahms et al. 1966; Piazzolla et al. 1977b). The data are shown in Fig. 6. No clear evidence of curvature is obtained suggesting that the thermal denaturation of both virus nucleoprotein and its RNA moiety shows little cooperativity. This also indicates that the base pairing does not involve long double-helical sequences. Calculation of apparent enthalpy and entropy changes was not attempted owing to some scattering of the experimental points. However, we simply note here the large difference in slope between the lines for virus nucleoprotein at low and high ionic strength, the behaviour of TYMV-RNA and TYMV nucleoprotein, at the same salt molarity, being closer to one another.

Fig. 7 shows the influence of the temperature on the intensity of the negative band at about 213 nm for T component. A conformational transition starting below 50 °C is
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Fig. 7. CD spectra of TYMV capsids in the far ultraviolet range at 30 °C (—), 50 °C (-----) and 80 °C (———). The insert shows the change in the 213 nm band as a function of temperature.

observed; apparently the increase in temperature induces an increase in secondary structure. Interestingly, the transition occurs in the same temperature range where it is believed that some dissociation of protein subunits takes place (Lyttleton & Matthews, 1958) under conditions of low ionic strength (0.1 M-NaCl).

DISCUSSION

The isolated virus nucleic acid has considerable base pairing which is not affected, at least in terms of overall secondary structure, by release from the virion. This was also found for μ2 phage (Isenberg et al. 1971) and R 17 phage (Hartman et al. 1973). Melting studies suggest the absence of long double-helical segments, characterized by cooperative behaviour, and that the total amount of base stacking plus base pairing is practically the same in RNA whether inside or outside the capsid. Thus, the general features of TYMV-RNA seem to be similar to those of chicory yellow mottle virus-RNA for which a structure formed by regions of single-chain stacked-base helices and by short double-helical loops has been proposed (Piazzolla et al. 1977b). As regards the amount of secondary structure present in TYMV-RNA, we recall that the Laser-Raman measurements of Turano et al. (1976) indicate about 60 % of base pairing and stacking. Matthews & Ralph (1966) estimated that the maximum possible pairing of complementary bases in TYMV-RNA is 78 %. The remainder is comprised almost exclusively of cytidylic acid residues and it is known
that poly (C) is in the form of single-chain stacked-base helices at neutral pH (Fasman et al. 1964; Maurizot et al. 1971). In view of the proposed model (Kaper, 1972; Jonard, 1972) of the protein–RNA linkage in TYMV based on hydrogen bonding between acidic amino acid and cytidine phosphate residues, the single-stranded segments of RNA possibly interact with protein in such a way that their stacking arrangement remains undisturbed.

Finally, the effect of ionic strength requires some comment. Fig. 5 shows that an increase of salt molarity induces a large decrease in the positive ellipticity of the virus nucleoprotein at low temperature and an elevation of the melting temperature. According to Jacrot et al. (1977), the high ionic strength could induce a swelling of the RNA allowing a stronger embedding of the nucleic acid into the capsid, thus contributing to the increase in the melting temperature and to the steeper slope of the van’t Hoff plot in Fig. 6. In addition, the well known stabilization of RNA structure at increasing ionic strength should also be considered.

The near ultraviolet CD spectrum of the capsid is rather complex (Fig. 2). Following the study of Budzynski (1971) on tobacco mosaic virus, the band at 293 nm can only be due to the tryptophanyl residue since only the indole chromophore absorbs significantly in this spectral region. No attempt is made to identify the inflection at 285 nm since at this wavelength both tyrosyl and tryptophanyl derivatives display CD bands. The 280 nm band can be assigned to the tyrosyl residue (Budzynsky, 1971 and references therein), the peak at 255 nm to phenylalanyl moieties (Horwitz et al. 1969) and the band at 240 nm to the tyrosyl residue as found for ribonuclease (Simons & Blout, 1968). It should be noted, however, that these are only tentative assignments, the confirmation of which should await a more detailed study. As regards the far ultraviolet range, the position of the positive band agrees with the wavelength of the $\pi-\pi^*$ transition of the $\beta$-structure, while that of the negative band does not correspond to any known polypeptide secondary structure. However, similar bands at 213 to 215 nm were also found in proteins shown to contain significant amounts of $\beta$-structure, such as ribonuclease (Tamburro et al. 1968a), pepsin (Tamburro et al. 1968b) and monellin (Jirgensons, 1976). A possible explanation might lie in the presence of distorted structures due to steric hindrance, causing a blue shift of the $\pi-\pi^*$ transition associated with the $\beta$-form (Schellman & Lowe, 1968). It should be appreciated, nevertheless, that the low values of the ellipticity of the extremes indicate that substantial portions of the proteins are in a non-periodic conformation. As shown in Fig. 2, comparison of the CD of the virus nucleoprotein and isolated RNA resulted in a different curve which did not match the CD of isolated capsids. More specifically, it can be said that the interaction with the nucleic acid induces a conformational change in the protein with a decrease of $\beta$-structure and some other structural variations as reflected by the changed environment of some aromatic residues, probably phenyalanines and tyrosines. Recent results of Piazzolla et al. (1977a) suggest the existence of some interference between protein–protein and protein–RNA bonds. Therefore, in the virus nucleoprotein, the disruption of some regions of $\beta$-structure is perhaps the energetic cost, in terms of intramolecular interactions, to be paid for attaining correct intermolecular protein–protein and protein–RNA stabilizing interactions.

When isolated capsids are heated in $0.1 \text{ M-NaCl}$, an increase of $\beta$-structure is observed. Interestingly, protein denaturation and aggregation is accompanied by a conformational change opposite to that induced in the virus nucleoprotein.
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REFERENCES


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