A STUDY OF THE INTERACTION OF TMV PROTEIN WITH SINGLE- AND DOUBLE-STRANDED POLYNUCLEOTIDES

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1. Introduction

The reconstitution of TMV in vitro is well established [1], but the mechanism of interaction of RNA and protein, especially at the stage of initiation remains unknown. It was shown that, in addition to TMV RNA, the virus protein formed the TMV-like complexes with certain polynucleotides, i.e. polypurines, poly(A) and poly(I) [2,3], as well as polypyrimidines, poly(U) and poly(C) [2]. However, the data concerning the interaction of the TMV protein with polypyrimidines were not confirmed by later studies [3,4] and this gave rise to the concept that TMV protein has a high affinity for purines, which, as was believed [5], could be realized at the initiation stage of the reconstitution of the virus. Our previous data [6,7] suggested that the interaction of polynucleotides with protein depended not only on the primary but also on the secondary structure of the polynucleotides. In the present work it is shown, with polynucleotides containing adenylic and uridylic acid residues, that the formation of double helices prevents the interaction of polynucleotides with TMV protein. At the same time, it is confirmed that TMV protein can interact with poly(U), but not poly(C), with a stable virus-like RNP particle being formed.

2. Materials and methods

TMV protein was prepared by the acetate method [8]. The preparations of poly(U) mol. wt. 800 000 (Novosibirsk) and mol. wt. 300 000 (Calbiochem), poly(C) (Sigma), poly(A), poly(A_{90}, U_{10}) and poly (A_{50}, U_{50}) (Reanal) were used. RNP was reconstituted from the protein and poly(U) (20 : 1 w/w), which was incubated in 0.1 M phosphate buffer, pH 6.6, for 18 h at 24°C. The mixture was dialyzed at 4°C against 0.05 M NaCl, 0.02 M Tris-HCl buffer, pH 7.2, for the night. RNP was sedimented by centrifugation at 4°C and purified by two or three reprecipitations. The concentration of polynucleotide in the RNP was determined by the total phosphorus content, the protein was determined by the Lowry method. In the study of TMV protein-polynucleotide interaction the components were mixed in a molar ratio of 1 to 3 (as judged by the monomer concentration in the polynucleotide) and incubated in 0.1 M phosphate buffer, pH 6.6, for 15 to 20 h at 24°C. The presence of the complex in the reaction mixture was inferred from the difference between the CD spectrum of the mixture and the sum of the spectra of the components, as described previously [9]. The CD spectra were taken in a Russel-Jouan II dichrograph. UV spectra were measured in a Cary-15 spectrophotometer. The figure shows the absorption spectra of RNP and repolymerized protein after the light scattering has been taken into consideration.

3. Results and discussion

Incubation of TMV protein with poly(U) in conditions optimal for interaction with poly(A) [3], i.e. 0.1 M phosphate buffer, pH 6.6, results in the formation of the complex. After dialysis of the reaction mixture in the cold against a neutral buffer, during which high molecular weight aggregates dissociate, the complex may be separated from the free compounds by centrifugation. The sedimentation

coefficient of the RNP in 0.1 M phosphate buffer pH 7.2, depends on the length of the polynucleotide, i.e. 50S in the case of poly(U) with mol. wt. 300 000 and 125S for a poly(U) with mol. wt. 800 000. In an acidic medium (pH 5.5) end-to-end aggregation of RNP seems to occur, and the sedimentation coefficients increase to 70S and 170S, respectively. It should be noted that the effectiveness of the interaction of TMV protein with poly(U), unlike that of poly(A), varied with different preparations of the protein in the range of 50 ± 30% (with poly(A): 90 ± 10%). Attempts at detecting a complex of TMV protein with poly(C) failed even when protein preparations most active with respect of poly(U) were used.

Fig.1 shows CD and u.v. absorption spectra of the RNP with poly(U). The CD spectrum in the near UV range has a positive band with a maximum at 269 nm and a small shoulder in the 287–292 nm range; the ellipticity in the maximum is 72200 ± 2000 per a nucleotide residue of poly(U). The CD spectrum of RNP does not change over a wide range of pH, from 5.5 to 9.5, testifying to the stability of the complex.

The CD curve of the bound form of poly(U) calculated as the difference between the spectrum of RNP and repolymerized protein, differs from the spectrum of the free polynucleotide in that the amplitude of the first positive band is much higher: $[\theta]_{max}$ – 59 000 ± 2000 and 16 700 ± 1000, respectively. The position of the maximum in the spectra of the free and bound forms is the same, i.e. 269 nm. A similar change in CD spectra was previously reported by us for intraviral RNA [6] and poly(A) and RNP [9]. The fact that the optical properties of bound forms of RNA, poly(A) and poly(U) are very close suggests that in all three the nature of the interaction of the protein and the polynucleotides is the same. As in the 260–280 nm region the CD bands of the nucleotide and protein chromophores overlap, it remains unknown what the contribution of the different constituents is.

An analysis of the u.v. spectra of RNP and its components has also revealed essential differences in the spectra of the bound and free forms of poly(U): the absorption of bound poly(U) decreases the maximum being still at 260 nm. As seen in fig.1C, the

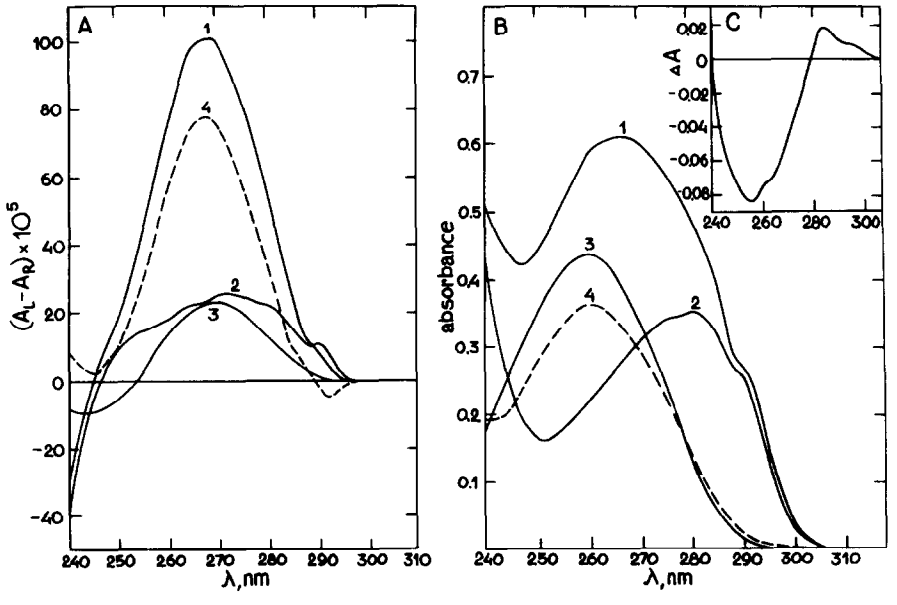

Fig.1. CD(A) and u.v.(B) spectra of RNP(1), repolymerized TMV protein (2), free (3) and intra-RNP (4) poly(U). Insert: u.v. difference spectrum (C) obtained by subtraction of (3) from (4). The spectra were measured in 0.1 M phosphate buffer, pH 5.5 (1,2) and 7.2 (3). The concentration of protein in (1,2) was 350 μg/ml, the concentration of poly(U) (P_i) in (1,3) was 4.61×10^{-5} M.

Table 1
Interaction of TMV protein with nucleotides

Polynucleotide	Concentration		$\Delta(\Delta\epsilon)_{max}$	$\Delta(A_L - A_R) \cdot 10^5$		Concentration of complex	Percentage incorporation
	polynucleotide	protein		270 nm	275 nm		
Poly(A)	6.6×10^{-5} M	2.2×10^{-5} M	10.3 ± 0.6[a]	50	64	6.28×10^{-5} M	95%
Poly(U)	6.6×10^{-5} M	2.2×10^{-5} M	16.4 ± 0.3	70.5	56.5	4.3×10^{-5} M	65%
Poly(A) · poly(U)	6.6×10^{-5} M	2.2×10^{-5} M		0	0	0	0
Poly(A_{90}, U_{10})	6.56×10^{-5} M	2.2×10^{-5} M	9.1 ± 0.3	43.5	57	6.26×10^{-5} M	95%
Poly(A_{50}, U_{50})	6.56×10^{-5} M	2.2×10^{-5} M		5.5	7		~10%

[a]The value of $\Delta(\Delta\epsilon)_{275}$ obtained in our experiments for RNP with poly(A) differs from that reported previously [9]. The reason for the discrepancy remains unknown.

difference spectrum has a complex pattern, i.e. the hypochromic effect is the highest in the 245–265 nm range, and in the longer wavelength region there is a small positive peak in the range of 280–305 nm. For a polynucleotide in a complex with protein, the alterations in the spectra could be produced, on the one hand, by destacking of the bases and, on the other, by higher hydrophobicity of the environment of the bases and their participation in the formation of hydrogen bonds with the protein. These concepts do not contradict what is known about the structure of TMV and may be used to explain the optical effects observed.

The effect of the secondary structure of the polynucleotide on the complex formation has been exemplified by the interaction of protein with an equimolar mixture of poly(A) and poly(U). With the same purpose, poly(A_{90}, U_{10}) and poly(A_{50}, U_{50}) copolymers were chosen, which differ in their ability to form intramolecular double helices.

In this series of experiments, the complexes were not isolated from the reaction mixture, and the interaction was estimated by the difference of the CD spectrum of the mixture from the sum of the spectra of the components in the region where this difference is most marked, i.e. 270–275 nm. The concentration of the complex was calculated with the help of difference coefficients of molar extinction, $\Delta(\Delta\epsilon)_{max}$ which were determined by a method previously described [9]. As seen from the data obtained (table 1), TMV protein readily forms complexes with single-stranded poly(A) and poly(U), but fails to interact with the same polynucleotides in the double-stranded form. Similar results were obtained with AU copolymers. The effectiveness of complex formation with poly(A_{90}, U_{10}) was similar to that of homopolynucleotides. Copolymer poly(A_{50}, U_{50}) assumes a double-stranded conformation and thus the degree of interaction decreases by approximately one order of magnitude.

The fact that TMV protein does not interact with double-stranded polynucleotides does not mean that it cannot unwind double helices. TMV RNA is known to have 60% of double-stranded fragments [10]; nevertheless, RNA is coated with protein in the course of virus formation. It seems that TMV protein may unwind double helices at the elongation stage of virus reconstitution while the initiation stage, as follows from the data obtained, requires the single-stranded conformation of the 5′-end of RNA, which makes it accessible to the protein. It is possible that it is this peculiarity of the secondary structure that is responsible for the failure to isolate the 5′-end fragment of RNA interacting with TMV protein by ribonuclease treatment [7,11]. The absence of specificity in the interaction of TMV protein with homopolynucleotides (according to our data, even such an 'unnatural' polynucleotide as poly(3,N^4-ethenocytidylic acid) incorporates into the complex) does not allow one to understand at the moment the principles of organisation of the primary structure of the single-stranded nucleotide sequence in the 5′-terminal region of TMV RNA.

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