A CIRCULAR DICHROISM STUDY OF THE INTERACTION OF ADENYLIC ACID OLIGO- AND POLYNUCLEOTIDES WITH TMV PROTEIN

T.P. LANINA, R.K. LEDNEVA and A.A. BOGDANOV

Laboratory of Bioorganic Chemistry, Lomonosov State University, Moscow, USSR

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1. Introduction

During TMV reconstitution, the specific protein–nucleic acid interaction is known to occur at the stage of initiation, i.e. when a complex is formed between the protein and the 5′-end of RNA. It is believed that subsequent formation of the protein coat does not depend on the primary structure of RNA [1]. As the formation of the initiation complex is the crucial moment of TMV self assembly, the reconstitution of the virus-like ribonucleoprotein complexes (RNP) involving short RNA fragments [2] and synthetic polynucleotides [3] may be regarded as a model of initiation allowing the problem of the recognition of nucleic acid by virus protein to be investigated with simple systems. A further development of this approach will be a study of the complex formation of the protein with oligonucleotides of a known structure.

The aim of the present work was to find out whether it is possible for TMV protein to form complexes with the adenylic series oligonucleotides in the conditions optimal for the interaction of TMV protein with poly(A) [3].

2. Materials and methods

The oligonucleotides were given to us by Dr. D.G. Knorre (Novosibirsk, Institute of Organic Chemistry). The poly(A) preparation (Reanal) was purified by phenol deproteinization. TMV protein was prepared by the acetic method [4]. The denatured protein was removed by centrifugation after being kept in 0.1 M phosphate buffer, pH 7.2 at room temperature for 30 min. The concentrations of the protein and oligo(poly)nucleotides were determined spectrophotometrically and the following coefficients were used: for protein – $A^{1\%}_{1cm}$ = 13 at 280 nm; for $(Ap)_2A$ and $(Ap)_3A$ – $\epsilon_{260,pH\,7.0}$ 12.8×10^3 and for $(Ap)_8A$, $(Ap)_9A$ and poly(A) – $\epsilon_{257,pH\,7.0}$ 10.1×10^3. The concentration of oligo(poly)nucleotides in RNP was expressed in moles of adenylic acid.

RNP was reconstituted from protein and poly(A) by the method of Fraenkel-Konrat and Singer [3]. To study the protein–oligonucleotide interaction, the components were mixed in a ratio of 1:3 (as calculated from the concentration of adenylic acid in the oligomers), because in the virus each protein subunit interacts with a trinucleotide RNA sequence. The samples were incubated in 0.1 M phosphate buffer, pH 6.6 at 30°C for 18–20 hr. Samples which contained the components of the mixture separately were treated in the same way. CD spectra were taken in a Jouan-II dichrograph at room temperature with the use of 1, 0.1 and 0.01 cm cells; in no case did the optical density of the solutions exceed 1.2. Ultraviolet spectra were taken in a Cary-15 spectrophotometer.

3. Results and discussion

Prior to studying the interaction of TMV protein with short oligonucleotides, RNP was reconstituted from poly(A) and virus protein. Then the RNP was used for determining the parameters of the spectrum of the bound form of adenylic acid and the optical effects accompanying complex formation.

The CD spectrum of RNP in the near ultraviolet region has a positive band with a maximum at 270 nm and a shoulder in the absorption region of trypto-

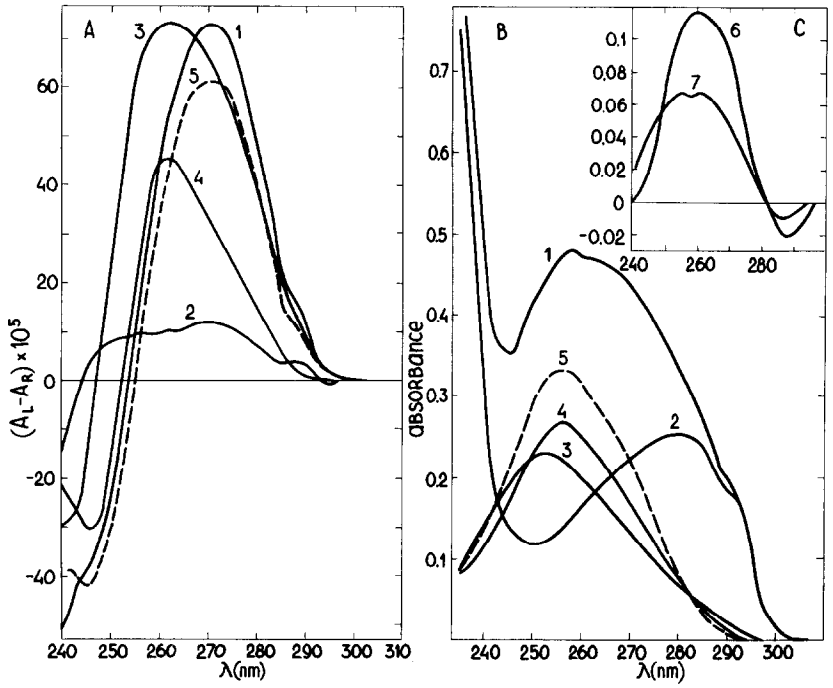

Fig. 1. CD(A) and ultraviolet(B) spectra of RNP (1), repolymerized TMV protein (2) free poly(A) (3, 4) and intra-RNP poly(A) (5). Insert: ultraviolet difference spectrum (C) obtained by subtraction of 3 from 1 (6) and of 4 from 2 (7). The spectra were measured in 0.1 M phosphate buffer, pH 5.5 (1–3), in 0.1 M phosphate buffer, pH 6.6 and 7.2 (4). The CD and ultraviolet spectra of RNP in buffers with pH 5.5, 6.6 and 7.2 are identical (1). The concentration of protein (measured by the Lowry method) in (1, 2) was 0.27 mg/ml, the concentration of poly(A) in (1, 3–5) was 2.7×10^{-5} M. The ultraviolet spectrum (1, 2) was corrected for light-scattering.

phan (285–290 nm); the ellipticity at 270 nm is 90 500 per poly(A) nucleotide residue. The CD spectra of RNP in 0.1 M phosphate buffer in the 5.5–7.2 pH range are identical (fig. 1). The spectrum of poly(A) in the complex was inferred from the difference between the spectra of RNP and repolymerised protein. The CD curve of the bound form of poly(A) differs from those of free poly(A) taken in 0.1 M phosphate buffer, pH 5.5–7.2. The CD spectra of poly(A) at pH 6.6 and 7.2 are similar both with regard to the position of the maximum (262 nm) and the magnitude of the amplitude ($[\theta]_{262}$ 56 500) of the first positive band, i.e. the structure of poly(A) at pH 6.6–7.2 does not change and the polynucleotide has a single-stranded conformation. When the pH is lowered to 5.5, the position of the maximum in the spectrum is the same but its amplitude increases ($[\theta]_{262}$ 91 000), due to double-stranded poly(A) being formed. Incorporation of polyA into RNP is accompanied by a red shift of the maximum of the first positive CD band from 262 to 270 nm; its amplitude ($[\theta]_{270}$ 75 500) increases as compared to that in the CD of poly(A) at pH 6.2–7.2 but is still lower than that at pH 5.5. The ultraviolet spectrum calculated for poly(A) inside RNP also differs from that of poly(A) in solution. The difference spectrum of the bound and free polynucleotide reveals a stronger absorption region with a maximum at 260 nm and a weaker absorption region at 287.5 nm. A higher optical density of the absorption band of adenosine is in agreement with the concept of the lack of base stacking in intravirus RNA [5]. The hypochromic effect in the 280–295 nm region may be the result of the change in the absorption of the chromophore group of the protein, most probably tryptophan, due to the formation of the complex with the polynucleotide.

It should be noted that similar changes in CD and ultraviolet spectra of TMV RNA occur on the forma-

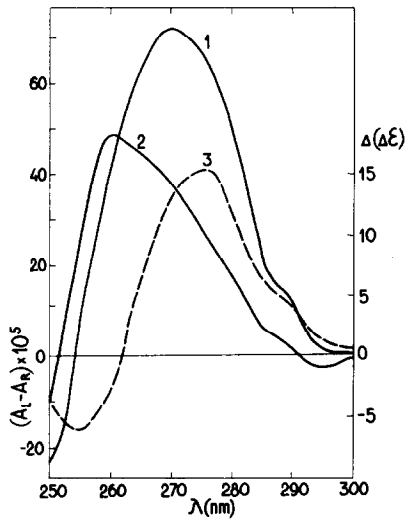

Fig. 2. CD spectra of RNP (1), of the algebraic sum of poly(A) and protein (2) and the difference CD spectrum (3) obtained by subtraction of 2 from 1. Measurements were made in 0.1 M phosphate buffer, pH 6.6 at a concentration of protein of 0.27 mg/ml (determined by the Lowry method) and of poly(A) 2.7×10^{-5} M.

tion of the virus: the maximum of the first positive CD band is shifted from 262 to 271 nm and the ellipticity increases from 24 100 to 54 800; the hyperchromic effect is 20–23% [6]. These data mean that the character of the changes in the structure of the polynucleotide on its being complexed with the virus protein and the mode of the interaction of protein with adenylic acid in RNP are generally the same as the interaction between the protein and nucleotide in TMV RNA.

The decomposition of the CD spectrum of RNP into those of the free components in 0.1 M phosphate buffer, pH 6.6 and comparison of the algebraic sum of the latter with the initial RNP spectrum allowed us to form an opinion about the changes in the optical properties of the system which accompany the interaction of the poly- and oligonucleotides of adenylic acid with protein (fig. 2). One can see that complex formation is followed by an increase in the CD in the 260–295 nm region, having a maximum at 276–277 nm; in the short wavelength range a band appears of the opposite sign. The CD difference referred to the concentration of one of the components (nucleotide was chosen) is a direct measure of the concentration of complex ($\Delta(\Delta\epsilon)_{275}$ 15.1).

Comparison of the CD spectra of the reaction mixture of oligonucleotides of $(Ap)_2A$ and $(Ap)_3A$ with the protein and the sum of the spectra of the components did not reveal any differences to exist between them when the concentrations of the protein and oligomers were 2×10^{-4} M and 6×10^{-4}, respectively. A considerable increase in the concentration of oligomers (to 7×10^{-3} M) did not lead to a positive result.

In the case of $(Ap)_8A$ and $(Ap)_9A$ the spectrum of the mixture was additive if the concentration of the protein and oligonucleotides were 1.8×10^{-5} and 5.7×10^{-5} M, respectively. When the concentrations were raised by one order of magnitude the spectrum lost its additive character, and the concentration of the complex was 5.1×10^{-5} M, which corresponded to 10% of the oligonucleotides being complexed. When the concentration of the protein in the sample was still higher and there was a 4-fold excess of oligonucleotides (4.8×10^{-4} M and 5.2×10^{-3} M, respectively), the concentration of the complex was 2.8×10^{-4} M, which meant that 20% of oligonucleotides had complexed. In the protein–poly(A) system, the concentration of the complex was 4×10^{-5} M and 4×10^{-4} M, if the concentrations of the protein and the polynucleotide were 1.8×10^{-5} M and 5.4×10^{-5} M, respectively, in one series of experiments and 1.8×10^{-4} M and 5.4×10^{-4} M in another series, which corresponds to 74% of complexed poly(A). The percentage of the complexed polynucleotide did not change if the poly(A)–protein ratio was increased up to 27: at concentrations of the protein and poly(A) of 2.4×10^{-4} M and 6.6×10^{-3} M, respectively, the concentration of RNP in the reaction mixture was 5.4×10^{-4} M. It seems that the complex formed in the mixture of protein and short oligonucleotides is rather unstable and is in a dynamic equilibrium with the free components; when the concentration of one of the two complex-forming substances is raised, the equilibrium shifts towards complex formation. With poly(A), the complex is stabilized by the multiple interactions of the protein with the polynucleotide, and its concentration, unlike that of the labile complex of the first type, is determined by the growth of the nucleoprotein helix rather than by initiation. It should be noted, that both with poly(A) and oligonucleotides, the shapes of the difference spectra are similar to that of the reconstructed spectrum shown in fig. 2, i.e. there is always a maximum at 276–277 nm and a

shoulder at 285–290 nm. Some deviations towards lesser effects were observed in both cases in the 260–270 nm range. This testifies to the fact that the short oligonucleotides in the complex have a single-stranded helical conformation.

Thus the use of $(Ap)_8A$ and $(Ap)_9A$ allowed the formation of complexes between the protein and short oligonucleotides to be demonstrated. According to the CD data, the general character of the interaction of protein with adenylic acid residues in the complex is not different from that of interaction with RNA nucleotides in the intact virus. Moreover, the experimental results shown above suggest that the specific nucleotide sequence near the 5′-end of TMV RNA, which is responsible for the initiation of virus reconstitution, may be rather short.

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