A compact form of double-stranded RNA in solutions containing poly(ethyleneglycol)

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ABSTRACT

Molecules of single-stranded ribosomal RNA and double-stranded replicative form of phage f2 RNA (dsRNA) adopt a compact form in solutions, containing sufficiently high concentrations of salt (NaCl) and polymer (PEG). However, only in the case of native dsRNA molecules the compact particles are characterized by a regular internal structure, which accounts for the appearance of an intense positive band in CD spectra. Heating or acidification of PEG-containing solutions of dsRNA leads to the disappearance of the intense positive CD band, which results from the "destruction" of the regular internal structure of compact particles. Comparison of properties of DNA and dsRNA compact particles formed in PEG-containing water-salt solutions suggests the existence of similar mechanisms of compactization of double-stranded polynucleotides.

INTRODUCTION

It has been shown¹⁻³ that native double-stranded DNA molecules in water-salt solutions, containing poly(ethyleneglycol) (PEG) can adopt a compact form (the so-called Ψ -form), which is characterized by a regular spatial arrangement of DNA chains. Due to a specific type of ordering of DNA chains within compact particles an intense negative band at 260-270 nm appears in their CD spectra¹⁻³. It is known also⁴ that a non-intercalating antibiotic distamycin A, when complexed with DNA prior to compactization, does not seem to change the mode of DNA packing in compact particles, although in this case an intense positive band at 265-270 nm (instead of a negative one) appears in CD spectra. Despite the possibility of theoretical explanations of the appearance of intense bands with different signs in CD spectra^{5,6}, the correlation between the secondary structure of polynucleotides, forming compact particles, and the type of their CD spectra is not fully understood.

In the present investigation we compared optical and X-ray diffraction characteristics of compact particles, formed from two types of high molecular weight RNA: a replicative double-stranded form of phage f2 RNA (dsRNA) and

single-stranded ribosomal RNA (rRNA). dsRNA, being similar to DNA in some structural parameters, belongs in contrast to DNA not to the B, but to the A family of polynucleotides.

MATERIALS AND METHODS

To prepare dsRNA^{*)} a logarithmically-growing culture of *E. coli* AB 105⁷ in tryptone-yeast extract medium supplemented with L-methionine (50 μ g/ml), was infected with phage f2 sus II (10-20 plaque-forming units per bacterium⁸) and cultivated overnight at 34°C on a Dubnoff shaker. DNA and ds RNA were extracted according to Marmur⁹. To remove host nucleic acids crude RNA, dissolved in 0.1 M NaCl + 10 mM MgCl₂ (about 5 mg/ml of nucleic acids), was treated with pancreatic DNase and RNase (10 μ g/ml of each) for 30 min at room temperature with stirring. The enzymes were inactivated by adding bentonite (1 mg/ml) and sodium dodecyl sulfate (0.1%), followed by phenol treatment. Degraded host nucleic acids were removed by repeated precipitation with isopropanol as indicated⁹. Traces of polysaccharidic material were removed using the potassium phosphate-2-methoxyethanol extraction step as described by Kirby¹⁰.

The pure dsRNA, dissolved in 0.1 SSC, had a melting temperature $T_m = 87^\circ\text{C}$, while no change of absorbance was observed upon heating between 25 and 70°C; the mean sedimentation coefficient s_w^{20} was 10.6. According to electron-microscopic observations (Fig. I) the preparation of dsRNA consisted mainly of double-stranded molecules with the mean length of $1/\mu$ (which corresponds to molecular weight of approximately 2×10^6 daltons) and also of a small amount of shorter molecules. When examined by acrylamide gel electrophoresis¹¹ the dsRNA was also shown to consist of integral duplex phage genomes (molecular weight $\sim 2.2 \times 10^6$ daltons), accompanied by small amounts of shorter molecules, forming discrete bands at higher electrophoretic mobilities¹².

Preparation of RNA from rat liver polysomes was kindly given to us by E.V. Brikina (Institute of Medical Biology, AMS USSR). The preparation consisted of two fractions with sedimentation coefficients of 18S and 28S.

Heat denatured dsRNA was prepared by heating a water-salt solution of dsRNA (5×10^{-3} M phosphate buffer + 10^{-4} M EDTA; $C_{\text{RNA}} \sim 20 \mu\text{g/ml}$) for 10 min at 100°C. After quick cooling of this solution in an ice bath the remaining

^{*)} dsRNA was prepared in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Fig.1. Electron micrograph of dsRNA dissolved in 0.3 M NaCl + 0.01 M phosphate buffer, pH 6.8. $C_{\text{RNA}} = 10 \mu\text{g/ml}$.

hyperchromicity of dsRNA was about 17%. Then the ionic strength of the solution was raised up to 0.4 by the addition of 4 M NaCl.

The preparation of PEG (molecular weight 20,000; "für die Gas-Chromatographie", "Merck", West Germany) was used without additional purification. Initial water-salt solutions, as well as those, containing PEG, were filtered through membrane filters "Synpor" ("Chemapol", Prague; diameter of the pores - 1.5μ).

Concentrated stock solutions of dsRNA were centrifugated for 1 h at 6,000 rev/min to remove mechanical impurities.

The method for preparation of RNA compact particles was similar to that described in detail for DNA¹³. Compact RNA particles were formed by mixing equal volumes of RNA and PEG solutions, both containing the desired concentration of NaCl and 0.01 M phosphate buffer, pH 6.8 unless otherwise stated. After shaking the mixture for 5 min it was left at room temperature for 12 h. RNA concentration in PEG-containing solutions did not exceed $25 \mu\text{g/ml}$.

PEG-containing water-salt solutions of RNA at different pH values were prepared by mixing water-salt solutions of RNA and PEG, both made up to the desired pH value (the procedure was described in detail earlier for DNA²).

The absorption and CD spectra of RNA solutions were recorded, using the "Beckman" model 25 spectrophotometer (USA) and the "Roussel-Jouan" CD-I85 dichrograph (France), respectively.

Preparation of RNA samples (from polymer-free water-salt solutions of RNA and from those, containing PEG) for electron microscopy was as follows. RNA was deposited on carbon-coated grids by touching with them the surface of RNA-containing solution ($C_{\text{RNA}} \sim 10 \mu\text{g/ml}$). The excess solution was removed from the grids with a filter paper. After washing, RNA samples, prepared from polymer-free solutions, were rotatory shadowed at an angle of about 6° with Pt/Pd alloy, while samples of RNA compact particles were contrasted with uranylacetate (1% solution). RNA samples were studied using an electron microscope JEM -100-B (Japan).

Samples of RNA compact particles were prepared for X-ray analysis as described earlier³. RNA compact particles were formed in 60-70 ml of a solution, containing RNA in concentration of about $20 \mu\text{g/ml}$. The formation and properties of compact particles were controlled by recording absorption and CD spectra. Then compact particles were concentrated by low-speed centrifugation (6,000 rev/min; 40 min). The sediment with a droplet of mother liquor was placed in a quartz capillary and centrifugated once more. Dense pellets of RNA compact particles covered with a thin layer of mother liquor and sealed in quartz capillaries were used as samples for X-ray analysis. X-ray powder patterns from these samples were taken with a camera, which permitted to obtain small- and wide-angle patterns simultaneously¹⁴. $\text{CuK}\alpha$ radiation and a nickel filter were used. The specimen-film distance was 200 mm, exposure times ranged between 24 and 36 h.

RESULTS AND DISCUSSION

Compactization of ribosomal RNA. Fig.2 shows absorption spectra of rRNA water-salt solutions (0.1 - 1 M NaCl), containing PEG ($C_{\text{PEG}} = 150 \text{mg/ml}$). One can see, that at NaCl concentrations, exceeding a "critical" value (0.3 M; curve 3), absorption spectra of rRNA differ from that of rRNA in polymer-free water-salt solution (curve 1). The differences consist in a small decrease of the amplitude of the absorption band at 257 nm, in a "red" shift of the maximum of this band and in the appearance of an "apparent" optical density at $\lambda > 320 \text{ nm}$. It can be seen that the absorption spectra are practically independent of NaCl concentration when it exceeds its "critical" value. The character of the observed changes in rRNA absorption spectra indicates, that at $C_{\text{NaCl}} \geq 0.3 \text{ M}$ rRNA molecules take the form of large particles,

Fig.2. Absorption spectra of rRNA: I - in a polymer-free solution; 2-4 - in the presence of PEG ($C_{\text{PEG}}=150$ mg/ml). C_{NaCl} (M): 2 - 0.1; 3 - 0.3; 4 - 1.

which scatter the UV light¹⁵⁻¹⁷.

Fig.3 shows the CD spectra of rRNA water-salt solutions, containing PEG ($C_{\text{PEG}}=150$ mg/ml), at different NaCl concentrations. It can be seen that the increase of NaCl concentration in a solution causes only minor changes in the CD spectrum, characteristic of the A form of rRNA (curve I). At high NaCl concentrations (when, as judged by the absorption spectra (Fig.2), rRNA molecules undergo a transition to a compact form) the amplitude of the positive CD band at 265 nm decreases and its maximum is "red"-shifted. These alterations of the CD spectra are in accord with the influence of light scatter by large rRNA particles¹⁷⁻¹⁹.

Wide-angle X-ray diffraction patterns of rRNA particles, formed in PEG-containing solutions, show reflections at spacings of 2.1; 3.0 and 3.8 Å, the values of which suggest that rRNA molecules in the compact form possess the secondary structure, which belongs to the A family. The absence of any reflections in small-angle patterns of these samples indicates that the folding of polynucleotide chains in rRNA particles lacks any regularity.

Compactization of double-stranded RNA. Fig.4 shows absorption spectra of dsRNA water-salt solutions, containing different concentrations of PEG.

Fig.3. CD spectra of rRNA in a polymer-free solution and in the presence of PEG ($C_{\text{PEG}}=150 \text{ mg/ml}$) at different ionic strengths. The curves are the same as in Fig.2.

Fig.4. Absorption spectra of dsRNA water-salt solutions ($C_{\text{NaCl}}=0.5 \text{ M}$). C_{PEG} (mg/ml): 1 - 0; 2 - 20; 3 - 40; 4 - 60; 5 - 100; 6 - 150.

One can see that at PEG concentrations, exceeding the "critical" value (which is about 60 mg/ml for the solution with ionic strength of 0.5), absorption spectra differ significantly from that of dsRNA in polymer-free solutions (curve I). Similar to the case of rRNA a decrease of the amplitude of the absorption band at 257 nm, a "red" shift of its maximum and the "apparent" optical density demonstrate that dsRNA molecules form large particles, which scatter the UV light¹⁵⁻¹⁷.

Thus from the data, described above, we may infer, that molecules of high molecular weight RNA under the influence of salt and polymer (PEG) adopt a form of particles, which, being analogous to DNA compact particles, formed in the presence of PEG¹⁵, may be called RNA compact particles.

Comparison of absorption spectra of dsRNA in PEG-containing solutions of different ionic strengths (0.3 - 1) shows that the "critical" concentration of PEG decreases with an increase of the ionic strength. For example, at $C_{NaCl} = 0.3$ M the "critical" value is 80 mg/ml, while at $C_{NaCl} = 0.5$ M the "critical" value is 60 mg/ml.

Fig.5 shows CD spectra of dsRNA water-salt solutions, containing different concentrations of PEG. It can be seen, that an increase of PEG con-

Fig.5. CD spectra of dsRNA water-salt solutions ($C_{NaCl} = 0.5$ M) at different PEG concentrations. The curves are the same as in Fig.4. 1-3 - left-hand ordinate; 4-6 - right-hand ordinate.

centration above the "critical" value causes substantial changes in the shape of the CD spectrum, characteristic of dsRNA in the absence of PEG (curve 1). The main features of the observed spectra (curves 4-6) are a large increase of the amplitude of the positive band, a shift of its maximum to 270 nm and an appearance of a small negative band in the region of 310 nm. Further increase in the PEG concentration results in the enhancement of the amplitude of the positive band ($\Delta\epsilon_{270} = 100$ at $C_{\text{PEG}} = 150$ mg/ml), while at high PEG concentrations the negative band at 310 nm disappears (curve 6).

From the data, presented in Fig.6, it can be inferred, that at a sufficiently high PEG concentration ($C_{\text{PEG}} = 150$ mg/ml) the increase of the ionic strength of dsRNA solutions has a negligible effect on the shape of the CD spectra.

Fig.6. CD spectra of dsRNA water-salt solutions, containing PEG ($C_{\text{PEG}} = 150$ mg/ml). C_{NaCl} (M): 1 - 0.1; 2 - 0.3; 3 - 0.5; 4 - 1. 1 - left-hand ordinate; 2-4 - right-hand ordinate.

It has been shown also (Fig.7) that the value of $\Delta\epsilon$ is essentially independent of the initial concentration of dsRNA (ranging from 2.5 to $30 \mu\text{g/ml}$), which was used for preparation of compact particles.

It should be pointed out that the observed transition of dsRNA to a compact state, characterized by specific optical properties, has proved to

Fig. 7. The dependence of the amplitude of the positive CD band ($\Delta\epsilon_{270}$) on dsRNA concentration in a solution. $C_{NaCl}=0.3$ M; $C_{PEG}=120$ mg/ml.

Fig. 8. CD spectra of dsRNA solutions ($C_{NaCl}=0.3$ M): 1 - in the absence of PEG; 2 - in the presence of PEG ($C_{PEG}=120$ mg/ml); 3 - solution 2 after a three-fold dilution with respect to PEG and dsRNA. 1, 3 - left-hand ordinate; 2 - right-hand ordinate.

be fully reversible. It can be seen (Fig.8), that when dsRNA solution, containing PEG ($C_{\text{PEG}}=120$ mg/ml) (curve 2) is diluted with a water-salt solution to the final PEG concentrations below the "critical" value (e.g., 40 mg/ml), the CD spectrum becomes identical with that of dsRNA in the absence of PEG (curves I and 3).

Acidification of a PEG-containing solution of dsRNA leads to a decrease of the positive CD band (Fig.9). It disappears at such pH values, which bring about the acid denaturation of the initial dsRNA. It should be stressed that the positive CD band disappears despite the fact that dsRNA molecules at these low pH values still retain the form of compact particles.

Fig.9. The dependence of the amplitude of the positive CD band ($\Delta\epsilon_{270}$) of dsRNA compact particles on pH of solutions. $C_{\text{NaCl}}=0.3$ M; $C_{\text{PEG}}=90$ mg/ml.

Compact particles, formed in PEG-containing solutions from partially heat denatured dsRNA molecules (see "Materials and Methods") show a CD spectrum, lacking an intense positive band (Fig.10).

Thus we may infer, that the native secondary structure of dsRNA is essential to the appearance of an intense positive band in CD spectra of dsRNA compact particles.

When a solution, containing dsRNA compact particles, is heated, the amplitude of the positive CD band decreases. The "melting" curve, obtained

Fig.10. CD spectra of dsRNA water-salt solutions ($C_{NaCl}=0.4\text{ M}$): 1 - native dsRNA in a polymer-free solution; 2 - heat denatured dsRNA (see "Materials and Methods") in the presence of PEG ($C_{PEG}=125\text{ mg/ml}$).

by taking CD spectra at different temperatures, shows, that the disappearance of the positive band occurs in a relatively narrow temperature interval (Fig.II). The resulting CD pattern resembles that of partially heat denatured dsRNA in PEG-containing solutions (Fig.I0, curve 2). However, the "melting" of the secondary structure of dsRNA under the conditions employed takes place at temperatures higher than 90°C , that is, far exceeding the temperatures, at which the CD transition occurs ($\sim 56^{\circ}\text{C}$). This fact suggests^{20,21} that the disappearance of the positive band reflects the "destruction" of the regular tertiary structure of dsRNA compact particles. The "destruction" temperature (T_m) of the tertiary structure of the particles, obtained from CD measurements, increases with the increase of the concentration of PEG in a solution (e.g., at the ionic strength 0.3 $T_m=56^{\circ}\text{C}$ at $C_{PEG}=90\text{ mg/ml}$, while at $C_{PEG}=150\text{ mg/ml}$ $T_m=90^{\circ}\text{C}$), but it never exceeds the "melting" temperature of the secondary structure of dsRNA. We may thus conclude that heating of a solution of dsRNA compact particles, similar to the case of DNA^{20,21}, brings about at first the destruction of the tertiary struc-

Fig.II. The dependence of the amplitude of the positive CD band ($\Delta \epsilon_{270}$) of dsRNA compact particles on the temperature of a solution. $C_{\text{NaCl}} = 0.3 \text{ M}$; $C_{\text{PEG}} = 90 \text{ mg/ml}$.

ture, with which the positive CD band is associated, and then the "melting" of the secondary structure of dsRNA. It should be mentioned also that at constant NaCl and PEG concentrations in a solution the "destruction" temperature of the tertiary structure of dsRNA compact particles is always about 10°C higher than that of DNA compact particles^{20,21}

Small-angle X-ray diffraction patterns of compact particles, formed in the presence of PEG from native dsRNA molecules, show one intense diffuse ring at a spacing of $35 \text{ \AA}$ (Fig.I2a). Wide-angle reflections (Fig.I2b) correspond to periods of 2.9; 3.9; 7.8 and $12.8 \text{ \AA}$, the values of which are close to those, characteristic of the A fiber structure. It should be pointed out that compact particles, formed from partially heat denaturated dsRNA molecules, lack not only an intense positive band in their CD spectra (Fig.I0) but also small-angle reflections in their X-ray patterns, which is indicative of an irregular type of folding of polynucleotide chains in such compact particles.

According to electron microscopic observations (Fig.I3) dsRNA compact particles, formed in PEG-containing solutions, have the form of disks or spheres $200\text{--}400 \text{ m}\mu$ in diameter. (It should be mentioned that no similar particles could be found in electron micrographs of control samples, prepared

Fig.12; X-ray diffraction patterns of dsRNA compact particles, formed in a water-salt solution, containing PEG. $C_{NaCl}=0.4$ M; $C_{PEG}=150$ mg/ml.

Fig.13. An electron micrograph of dsRNA compact particles, formed in a water-salt solution, containing PEG. $C_{NaCl}=0.3$ M; $C_{PEG}=100$ mg/ml.

from dsRNA solutions at C_{PEG} below the "critical" value (see above). In some cases (designated by arrows in Fig.13) it is clearly seen that the electron density in the central part of the particles is quite small. This fact may indicate that dsRNA molecules, being driven to a compact state, can take the form of toroids (similar to the case of DNA^{13,22}).

It is interesting to compare properties of compact particles, formed from DNA and RNA molecules in PEG-containing solutions. The first important observation is that only native double-stranded polynucleotides (DNA^{2,3} and

dsRNA) form compact particles with a regular internal structure, characterized by the same period of identity equal to 35 Å. One may thus suppose that the formation of a specific common tertiary structure of dsRNA and DNA compact particles is based upon similar features of the secondary structure of double-stranded polynucleotides.

As the periodicity of 35 Å is not characteristic of dsRNA secondary structure, the presence of this reflection in X-ray patterns of dsRNA compact particles lends further support to the hypothesis³ that this reflection, being not connected with the regularity of the secondary structure of polynucleotides, must be attributed to the so-called "microcrystalline" regions in compact particles^{2,3}.

The present investigation shows that the properties of dsRNA compact particles, formed in solutions, containing sufficiently high salt (NaCl) and polymer (PEG) concentrations, are very similar to those of the Ψ -form of DNA. Thus it is justified to apply the term " Ψ (polymer-salt-induced)"-form for designation of dsRNA compact particles. Taking into account the opposite sign of intense CD bands, characteristic of compact particles, formed from DNA molecules (being in the B form previous to compactization) and dsRNA molecules (being in the A form), one may infer the existence of two families (Ψ_+ and Ψ_-) of compact particles, differing by the secondary structure of polynucleotides. The earlier studies on compactization of DNA, complexed with distamycin A⁴, suggest the possibility of a continuous structural transition from one family to the other. It is possible that CD spectra, belonging either to Ψ_+ or to Ψ_- families, which were observed for complexes of different histones with DNA²³⁻²⁵, are indicative of different alterations of DNA secondary structure, brought about by histones.

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