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 repli-
cative 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 solu-
tions suggests the existence of similar mechanisms of compactization of
double-stranded polynucleotides.

INTRODUCTION

     It has been shown[1-3] 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 spectra[1-3]. It is known also[4] 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 I05[7] in tryptone-yeast extract medium supplemented with L-methionine (50μg/ml), was infected with phage f2 sus II (I0-20 plaque-forming units per bacterium[8]) and cultivated overnight at 34°C on a Dubnoff shaker. DNA and ds RNA were extracted according to Marmur[9]. To remove host nucleic acids crude RNA, dissolved in 0.I M NaCl + I0 mM MgCl$_2$ (about 5 mg/ml of nucleic acids), was treated with pancreatic DNAse and RNAse (I0μg/ml of each) for 30 min at room temperature with stirring. The enzymes were inactivated by adding bentonite (I mg/ml) and sodium dodecyl sulfate (0.I%), followed by phenol treatment. Degraded host nucleic acids were removed by repeated precipitation with isopropanol as indicated[9]. Traces of polysaccharidic material were removed using the potassium phosphate-2-methoxyethanol extraction step as described by Kirby[I0].

The pure dsRNA, dissolved in 0.I SSC, had a melting temperature $T_m =$ 87°C, while no change of absorbance was observed upon heating between 25 and 70°C; the mean sedimentation coefficient s_w^{20} was I0.6. According to electron-microscopic observations (Fig.I) the preparation of dsRNA consisted mainly of double-stranded molecules with the mean length of Iμ (which corresponds to molecular weight of approximately 2xI0^6 daltons) and also of a small amount of shorter molecules. When examined by acrylamide gel electrophoresis[II] the dsRNA was also shown to consist of integral duplex phage genomes (molecular weight $\sim$ 2.2xI0^6 daltons), accompanied by small amounts of shorter molecules, forming discrete bands at higher electrophoretic mobilities[I2].

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 I8S and 28S.

Heat denatured dsRNA was prepared by heating a water-salt solution of dsRNA (5xI0^{-3} M phosphate buffer + I0^{-4} M EDTA; $C_{RNA} \sim$ 20μg/ml) for I0 min at I00°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.
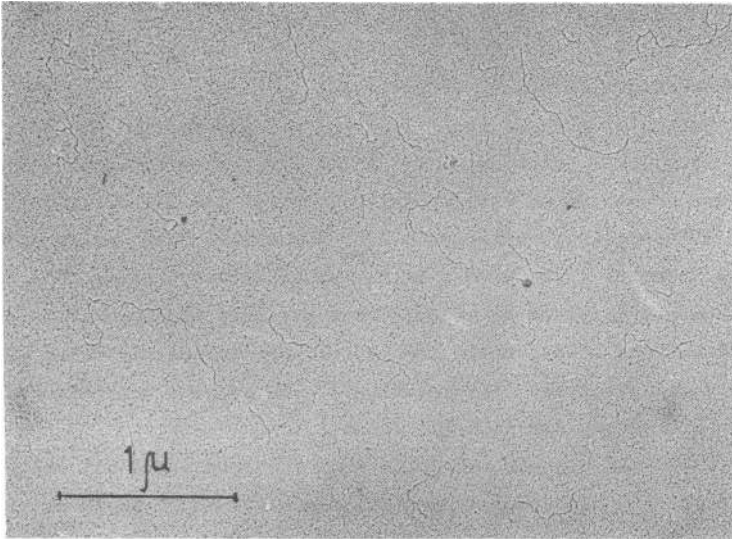
Fig.I. Electron micrograph of dsRNA dissolved in 0.3 M NaCl + 0.0I M phosphate buffer, pH 6.8. C_{RNA}=I0μg/ml.

hyperchromicity of dsRNA was about I7%. 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 - I.5μ).

Concentrated stock solutions of dsRNA were centrifugated for I 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 DNA[13]. Compact RNA particles were formed by mixing equal volumes of RNA and PEG solutions, both containing the desired concentration of NaCl and 0.0I M phosphate buffer, pH 6.8 unless otherwise stated. After shaking the mixture for 5 min it was left at room temperature for I2 h. RNA concentration in PEG-containing solutions did not exceed 25μg/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 DNA[2]).

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 ($C_{RNA} \sim 10 \mu g/ml$). 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 (I% solution). RNA samples were studied using an electron microscope JEM -I00-B (Japan).

Samples of RNA compact particles were prepared for X-ray analysis as described earlier[3]. RNA compact particles were formed in 60-70 ml of a solution, containing RNA in concentration of about $20 \mu g/ml$. 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 simultaneously[14]. CuK_{α} 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.I - I M NaCl), containing PEG (C_{PEG}=I50mg/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 I). 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 $\lambda > 320$ 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 $C_{NaCl} \geqslant 0.3$ M rRNA molecules take the form of large particles,
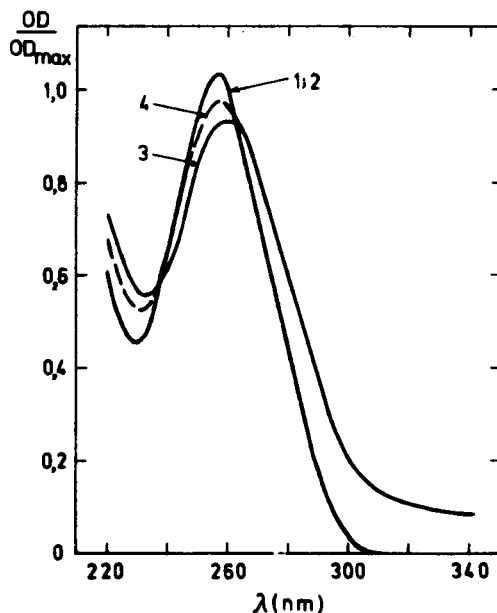
which scatter the UV light[I5-I7].

Fig.3 shows the CD spectra of rRNA water-salt solutions, containing PEG (C_{PEG}=I50 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 particles[I7-I9].

Wide-angle X-ray diffraction patterns of rRNA particles, formed in PEG-containing solutions, show reflections at spacings of 2.I; 3.0 and 3.8 A, 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.
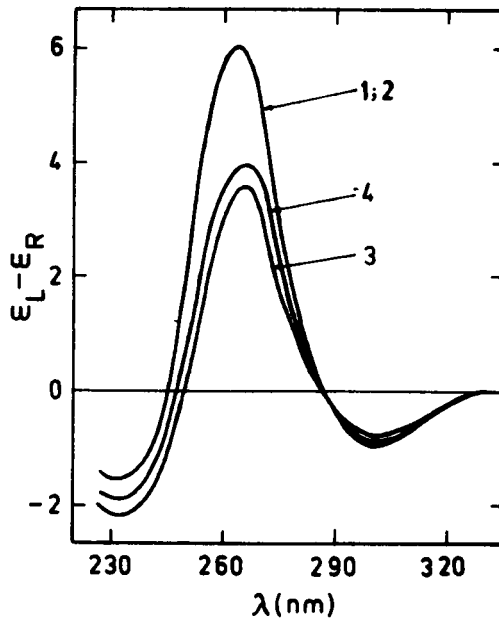
Fig.3. CD spectra of rRNA in a polymer-free solution and in the presence of PEG (C_{PEG}=150 mg/ml) at different ionic strengths. The curves are the same as in Fig.2.
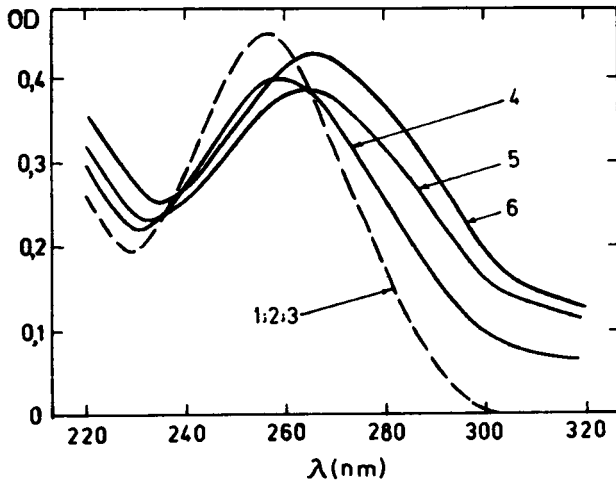

Fig.4. Absorption spectra of dsRNA water-salt solutions (C_{NaCl}=0.5 M). C_{PEG}(mg/ml): I - 0; 2 - 20; 3 - 40; 4 - 60; 5 - I00; 6 - 150.

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 amplit-
ude of the absorption band at 257 nm, a "red" shift of its maximum and the
"apparent" optical density demonstrate that dsRNA molecules form large par-
ticles, which scatter the UV light[15-17].

   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 PEG[15], may be called RNA compact particles.

   Comparison of absorption spectra of dsRNA in PEG-containing solutions
of different ionic strengths (0.3 - I) shows that the "critical" concen-
tration 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 dif-
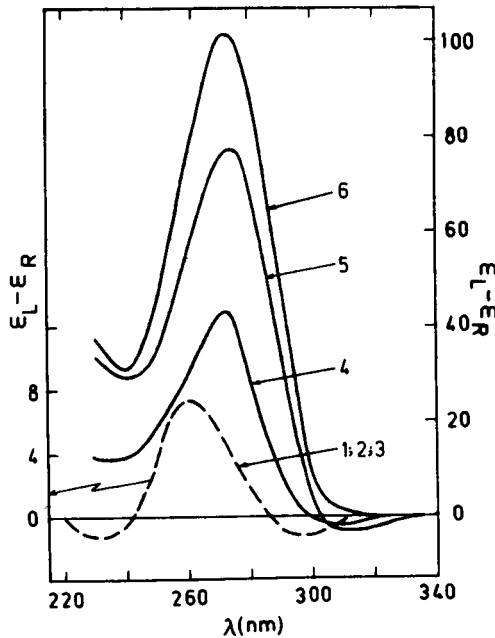ferent concentrations of PEG. It can be seen, that an increase of PEG con-

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. I-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 I). 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 max-
imum to 270 nm and an appearance of a small negative band in the region of
3IO nm. Further increase in the PEG concentration results in the enhancement
of the amplitude of the positive band ($\Delta\varepsilon_{270}$= IOO at C_{PEG}=I50 mg/ml), while
at high PEG concentrations the negative band at 3IO nm disappears (curve 6).

From the data, presented in Fig.6, it can be infered, that at a suffi-
ciently high PEG concentration (C_{PEG}=I50 mg/ml) the increase of the ionic
strength of dsRNA solutions has a negligible effect on the shape of the CD
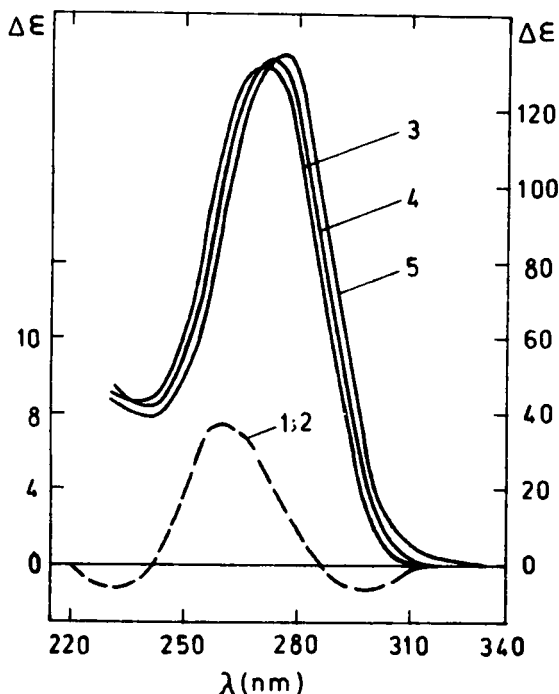spectra.

Fig.6. CD spectra of dsRNA water-salt solutions, containing PEG (C_{PEG}=
I50 mg/ml). C_{NaCl} (M): I - 0.I; 2 - 0.3; 3 - 0.5; 4 - I. I - left-hand
ordinate; 2-4 - right-hand ordinate.

It has been shown also (Fig.7) that the value of $\Delta\varepsilon$ is essentially
independent of the initial concentration of dsRNA (ranging from 2.5 to
30 μ 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
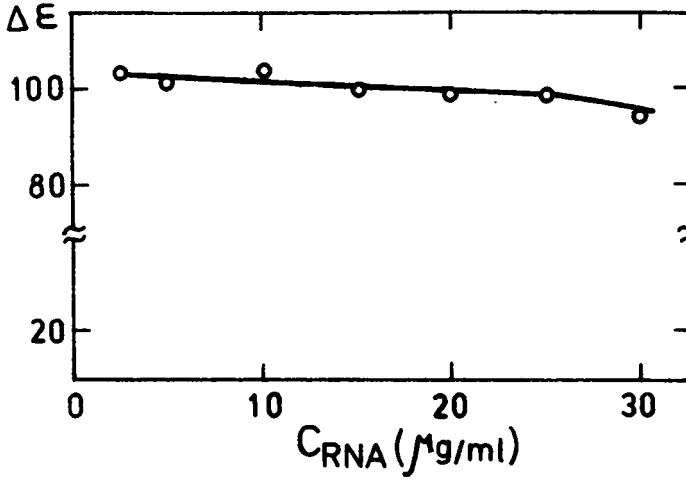
Fig.7. The dependence of the amplitude of the positive CD band $(\Delta \mathcal{E}_{270})$ on dsRNA concentration in a solution. $C_{NaCl} = 0.3$ M; $C_{PEG} = 120$ mg/ml.
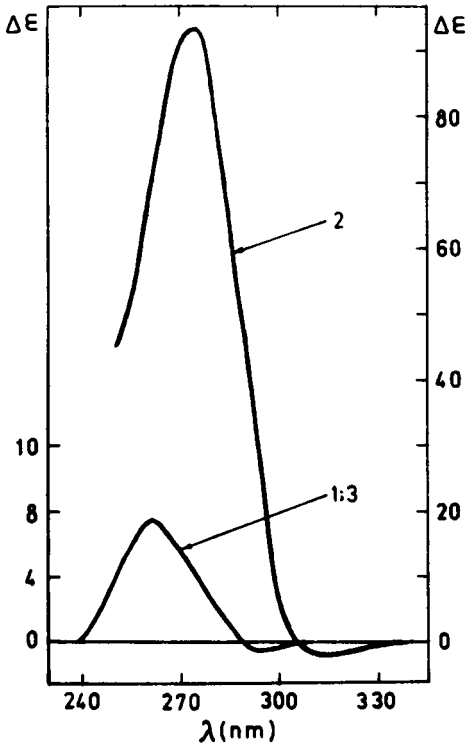

Fig.8. CD spectra of dsRNA solutions ($C_{NaCl} = 0.3$ M): I - in the absence of PEG; 2 - in the presence of PEG ($C_{PEG} = 120$ mg/ml); 3 - solution 2 after a three-fold dilution with respect to PEG and dsRNA. I, 3 - left-hand ordinate; 2 - right-hand ordinate.

be fully reversible. It can be seen (Fig.8), that when dsRNA solution, containing PEG (C_{PEG}=I20 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.
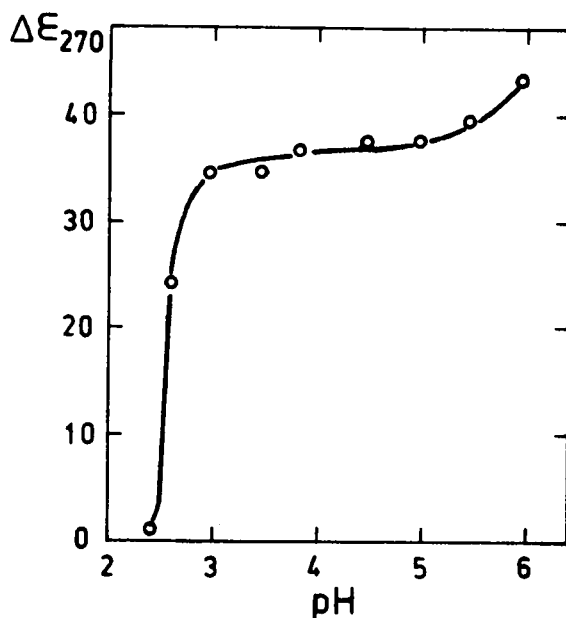

Fig.9. The dependence of the amplitude of the positive CD band ($\Delta\mathcal{E}_{279}$) of dsRNA compact particles on pH of solutions. C_{NaCl}=0.3 M; C_{PEG}=90 mg/ml.

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.IO).

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
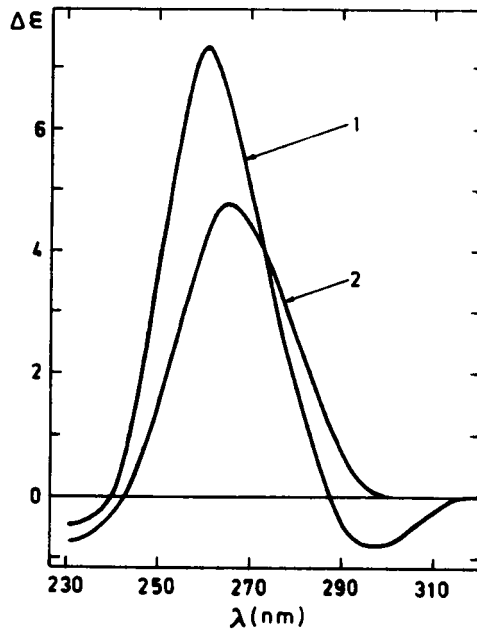
Fig.IO. CD spectra of dsRNA water-salt solutions (C_{NaCl}=0.4 M): I - native dsRNA in a polymer-free solution; 2 - heat denaturated dsRNA (see "Materials and Methods") in the presence of PEG (C_{PEG}=I25 mg/ml).

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 dena-turated dsRNA in PEG-containing solutions (Fig.IO, curve 2). However, the "melting" of the secondary structure of dsRNA under the conditions employed takes place at temperatures higher than $90^{\circ}C$, that is, far exceeding the temperatures, at which the CD transition occurs ($\sim 56^{\circ}C$). This fact sug-gests[20,2I] that the disappearance of the positive band reflects the "destruc-tion" of the regular tertiary structure of dsRNA compact particles. The "destruction" temperature (τ_m) of the tertiary structure of the particles, obtained from CD measurements, increases with the increase of the concen-tration of PEG in a solution (e.g., at the ionic strength 0.3 τ_m=56°C at C_{PEG}=90 mg/ml, while at C_{PEG}=I50 mg/ml τ_m=90°C), but it never exceeds the "melting" temperature of the secondary structure of dsRNA. We may thus con-clude that heating of a solution of dsRNA compact particles, similar to the case of DNA[20,2I], brings about at first the destruction of the tertiary struc-
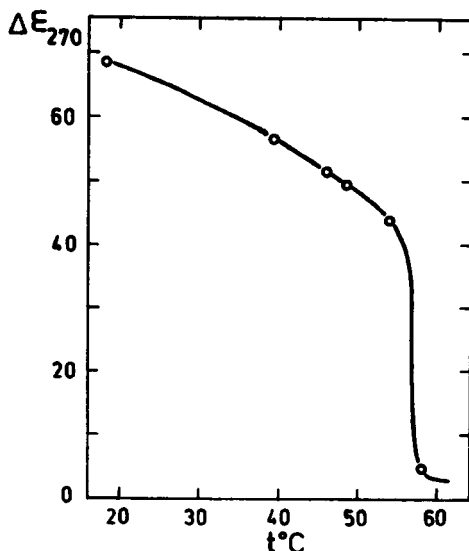
Fig.II. The dependence of the amplitude of the positive CD band ($\Delta\mathcal{E}_{270}$) of dsRNA compact particles on the temperature of a solution. C_{NaCl}=0.3 M; C_{PEG}=90 mg/ml.

ture, with which the positive CD band is assosiated, 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 A (Fig.I2a). Wide-angle reflections (Fig.I2b) correspond to periods of 2.9; 3.9; 7.8 and I2.8 A, 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.IO) 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 sphres 200-400 μ in diameter. (It should be mentioned that no similar particles could be found in electron micrographs of control samples, prepared
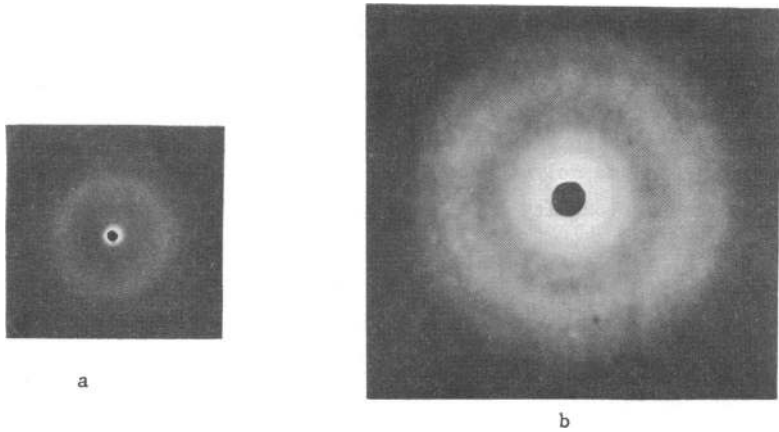
Fig.I2; X-ray diffraction patterns of dsRNA compact particles, formed in a water-salt solution, containing PEG. C_{NaCl}=0.4 M; C_{PEG}=I50 mg/ml.
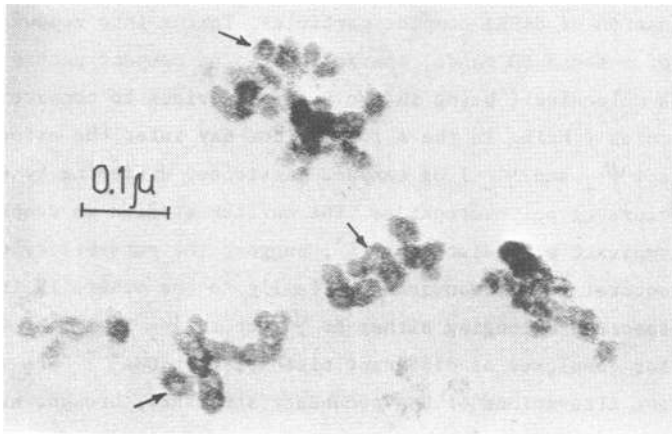

Fig.I3. An electron micrograph of dsRNA compact particles, formed in a water-salt solution, containing PEG. C_{NaCl}=0.3 M; C_{PEG}=I00 mg/ml.

from dsRNA solutions at C_{PEG} below the "critical" value (see above). In some cases (designated by arrows in Fig.I3) 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 obser-vation is that only native double-stranded polynucleotides (DNA[2,3] and

dsRNA) form compact particles with a regular internal structure, charac-
terized 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 com-
pact particles lends further support to the hypothesis[3] that this reflection,
being not connected with the regularity of the secondary structure of poly-
nucleotides, 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 compactiza-
tion of DNA, complexed with distamycin A[4], suggest the possibility of a
continuous structural transition from one family to the other. It is pos-
sible that CD spectra, belonging either to ψ_+ or to ψ_- families, which
were observed for complexes of different histones with DNA[23-25], are indica-
tive of different alterations of DNA secondary structure, brought about by
histones.

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