A comparative X-ray diffraction and circular dichroism* study of DNA compact particles formed in water-salt solutions, containing poly(ethylene glycol)*

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ABSTRACT

Comparative CD and X-ray diffraction studies of DNA compact particules which were obtained in PEG-containing water-salt solutions, have been carried out. Compact particules, formed from native DNA, produce a ψ CD spectrum (characterized by a negative band at $\lambda \sim 270$ nm) and a small-angle X-ray diffraction pattern, which shows two reflections: I at 34-40 Å and II at 80-90 Å (together with its second-order reflection). Compact particules, formed from DNA molecules with partially disordered secondary structure, do not produce the ψ CD spectrum and the reflection I, while the reflection II remains unchanged. It is suggested that the spacing of 34-40 Å is associated with a side-by-side packing of DNA fragments in "microcrystalline" regions in compact particules and that such "microcrystallization" accounts for the generation of the ψ CD spectrum.

INTRODUCTION

In the last few years reports have been made on the formation of a compact form of double-stranded DNA in water-salt solutions, containing different polymers which did not interact with DNA (PEG[1,2], polyacrilate[3]). It has been shown that DNA molecules, being in the compact form, differ from those in linear, "open", form by their hydrodynamic[1,4], biological[5] and optical[2,3,6] properties, in particular, by the appreciable optical density at $\lambda > 320$ nm in their UV-spectra[6] and by the presence of a negative band at $\lambda \sim 265$-280 nm in their CD spectra (the so-called ψ spectra)[2,3]. In accordance with the electron microscopic observations[2,7] DNA compact particles, formed in the presence of PEG, have the shape of discs or doughnuts about 1,000 Å in diameter. Using all these properties, characteristic of the DNA compact form, one can easily detect its formation in a solution.

It should be pointed out that many properties of the DNA compact particles, formed in PEG-containing solutions (e.g. the toroidal shape, specific CD spectra) are similar to those of DNA complexes with some polyamino-

acids[8-11] or histones[12,13]. For this reason one may suppose that the DNA compact particles, obtained in PEG-containing solutions, may be regarded as a model for DNA compact state in vivo (in phages or in chromosomes) and that the study of such particules may give information about possible types of packing of double-stranded DNA molecules.

Earlier it was suggested[3,14] that the appearance of a specific CD spectrum of native DNA in PEG-containing solutions was connected with a regular folding of DNA double helixes. Recently an X-ray diffraction study of the DNA compact form, obtained in the presence of PEG, has been carried out[15]. The small-angle X-ray scattering maximum at 38.2-25.3 Å, observed in this work, was attributed to the distance between the axes of DNA molecules, arranged parallel to each other in compact particules. However, no characteristics of these particules have been reported; at the same time the compactization conditions, used in this work, in particular, extremely high DNA concentrations ($C_{DNA} \sim 350\ \mu g/ml$) significantly differed from those, being optimum for CD studies ($C_{DNA} < 30\ \mu g/ml$).

In the present paper we report a comparative study of X-ray diffraction patterns and CD spectra of DNA compact particules, formed in PEG-containing solutions under similar conditions, which minimized the possibility of a non-specific intermolecular aggregation of DNA.

MATERIALS AND METHODS

E. coli DNA was kindly given by Prof. G.P. Georgiev (Institute of Molecular Biology, Moscow); *Tetrahymena pyriformis* DNA - by Prof. V.I. Vorobjov (Institute of Cytology, Leningrad); calf thymus DNA - by Prof. H.Venner (The Central Institute of Microbiology and Experimental Therapy, Jena). Highly polymerized preparations of DNA from chicken blood ("Reanal", Hungary) and ("Calbiochem", USA) were used without additional purification; DNA from cattle spleen (Olajne, USSR) was purified by pronase treatment[16] and chloroform-isoamyl alcohol precipitation[17]. The protein and RNA content in DNA preparations, used in this work, was determined by standard procedures[18,19] and did not exceed 1% and 0.1%, respectively. All DNA stock solutions were centrifuged at 105,000 g to remove possible high-molecular weight polysaccharide-like impurities and then dialized against a 0.3M NaCl solution. All DNA preparations were shown to be fully native by acid titration and heat denaturation criteria. *E. coli* DNA had a molecular weight of about 10^7; it was sheared in an ice bath, using an ultrasound desintegrator "УЗДН-1УЧ.2" (22 kc; the sonication time - 10-20 sec.). PEG prepara-

tions of molecular weights 20,000 (für die Gas-Chromatography, "Merck", West Germany) and 4,000 ("Schuchardt", München) were used. Before the mixing with DNA all water-salt solutions, as well as those, containing PEG, were passed through membrane filters "Synpor" ("Chemapol", Prague).

DNA compact particules were prepared by a standard procedure[7] - by the mixing of equal volumes of DNA and PEG solutions, both containing 0.3M NaCl and 0.0I M phosphate buffer, pH 6.8 unless specifically mentioned. After shaking the mixtures were left to stand for I2 h. at room temperature. The DNA concentration in PEG-containing solutions did never exceed 25 μg/ml. Compact particules were formed from native DNA molecules as well as from those with altered secondary structures by the following procedures.

I. The mixing of solutions, containing PEG and native DNA.

2. The mixing of solutions, containing PEG and heat denaturated DNA. (DNA was heated at I00°C for I5 min. and then cooled quickly to 0°C; the remaining hyperchromic effect was about I0%.)

3. a) The mixing of solutions, containing PEG and acid denaturated DNA; both solutions were acidified (0.I N HCl) to pH 2.3 before the mixing. b) The acidification of the mixture, containing DNA compact particules, which was prepared by the procedure (I), to pH 2.3.

4. The "condensation" of DNA in the absence of PEG by the acidification of the DNA solution ($C_{DNA} \sim$ I0 μg/ml; 0.3 M NaCl) to pH 2.0[20].

5. The precipitation of DNA in the absence of PEG with a twice volume of ethanol ($C_{DNA} \sim$ 50 μg/ml; 0.3 M NaCl).

In each case before the preparation of a sample for X-ray analysis absorption and CD spectra of compact particules were recorded, using the "Hitachi" spectrophotometer (Japan) and the "Roussel-Jouan" CD-I85 dichrograph (France), respectively.

In order to prepare a sample of DNA compact particules for X-ray diffraction studies one needs nearly as much as I mg of DNA. Since DNA concentrations in PEG-containing solutions were relatively low (see above), it was necessary to concentrate the compact particules from 60-70 ml of a solution. The concentration was achieved by centrifugation of the solutions, containing the compact particules, for 40 min. at 6,000 rev./min. The supernatant was decanted and the pellet of the compact particules (with a small amount of mother liquor) was placed in a quartz X-ray capillary (and centrifuged once more, if necessary). Dense pellets of DNA compact particules, thus obtained, covered with a thin layer of mother liquor, were used as samples for X-ray diffraction studies.

X-ray powder diffraction photographs were taken with an evacuated X-ray diffraction camera[21], using CuK_α radiation from a BSV-9 tube (2.5 ma; 50 kV) or from a BSV-10 tube (15 ma; 40 kV) and a nickel filter (some photographs were obtained without the filter). The beam was collimated to a spot about 150μ in diameter; the specimen-film distance was 150-200 mm. Exposure times ranged between 12 and 48 h.

Samples for electron microscopic studies were prepared as follows. DNA compact particules were deposited on the parlodion(1% solution)-coated grids by touching the surface of the PEG-containing solution with the grids. The grids were dried on filter paper and the samples were stained with 1% aqueous uranyl acetate (40 sec.). Electron micrographs were taken with a JEM 100 B electron microscope ("JEOL", Japan) at the voltage of 80 kV.

RESULTS AND DISCUSSION

Compact particules, formed from native DNA molecules. Fig.1 shows the CD spectra of PEG-containing solutions of *E.coli* DNA, together with the spectrum of a PEG-free DNA solution. One can see that on the addition of PEG to a solution of native DNA (the procedure (I), see Materials and Methods) the conservative PEG-free CD spectrum (curve 1) undergoes a change to a pattern (curves 2-5), which was designated by the term ψ [3]. Its characteristic feature is a negative band at $\lambda_{min} \sim$ 270-280 nm. It can be seen that the amplitude of this band varies with the molecular weights of DNA (compare curves 2 and 4) and of PEG (compare curves 4 and 5) as well as with the PEG concentration (compare curves 3 and 4), in accordance with results, obtained earlier[2,3,22]. Similar changes in the shape of CD spectra were observed on the addition of PEG to DNA from different sources (see Materials and Methods), the only difference being in the amplitude of the negative band.

At DNA concentrations (C_{DNA}=215-250μg/ml) about ten times those used in the CD studies ($C_{DNA} \sim$ 20μg/ml) a macroscopic precipitation of DNA usually occurs soon after the mixing with PEG. Nevertheless, solutions, containing such aggregates, also displayed ψ CD spectra, though with markedly diminished amplitudes of the negative band as compared to the case of a dilute DNA solution (the difference being possibly due either to the effect of light-scattering by the aggregates or to a high probability of a non-specific aggregation in concentrated solutions).

Fig.2 shows X-ray powder diffraction patterns, characteristic of the compact particules, which were formed from native *E.coli* DNA molecules by

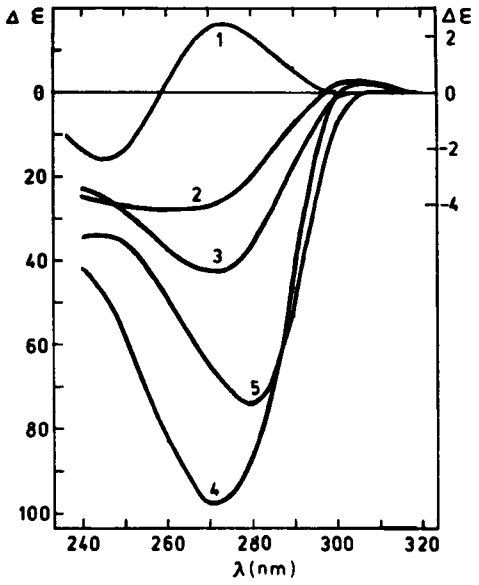


Fig.I. The CD spectra of native *E. coli* DNA in PEG-free and PEG-containing water-salt solutions, prepared by the procedure (I): I - in the absence of PEG, m.w.$_{DNA}$=1.3xIO^7; 2 - C_{PEG}=I50 mg/ml, m.w.$_{PEG}$=20,000, m.w.$_{DNA}$=7.4xIO^6; 3 - C_{PEG}=80 mg/ml, m.w.$_{PEG}$=20,000, m.w.$_{DNA}$=8.IxIO^5; 4 - C_{PEG}=I50 mg/ml, m.w.$_{PEG}$=4,000, m.w.$_{DNA}$=8.Ix IO^5; 5 - C_{DNA}=I50 mg/ml, m.w.$_{PEG}$=20,000, m.w.$_{DNA}$=8.IxIO^5.

the procedure (I), at C_{PEG}=I50 mg/ml, and characterized by the Ψ CD spectra (see Fig.I). It can be seen that the wide-angle pattern (Fig.2a) shows a set of diffuse rings at the spacings of 3.4; 4.3; 5.6; 8.6 and I2.8 Å, while the small-angle patterns (Fig.2b-d) show two reflections at d_I=34-37 Å (the reflection I) and at d_2=80-90 Å (the reflection II) (together with the second- and the third-order reflections). It must be pointed out that relative intensities of the reflections I and II may vary for different preparations of *E. coli* DNA: as a rule, they are either comparable (Fig;2b) or the intensity of the reflection I is greater than that of the reflection II (Fig. 2c). For one preparation the ring II appeared to be even more intense and sharp than the ring I (Fig.2d). (The reasons of such variations of the intensities were not studied in this work.) Sometimes the pushing of a pellet of DNA compact particules through a capillary resulted in its partial orientation. Then the 3.4 Å reflection became parallel to the axis of orientation, while the small-angle reflections I and II were found near the equator (Fig.

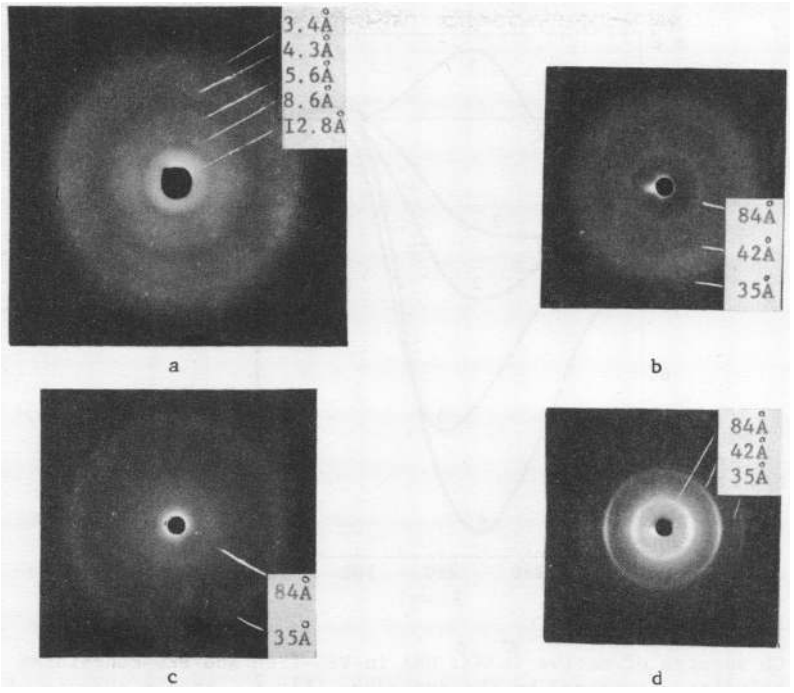
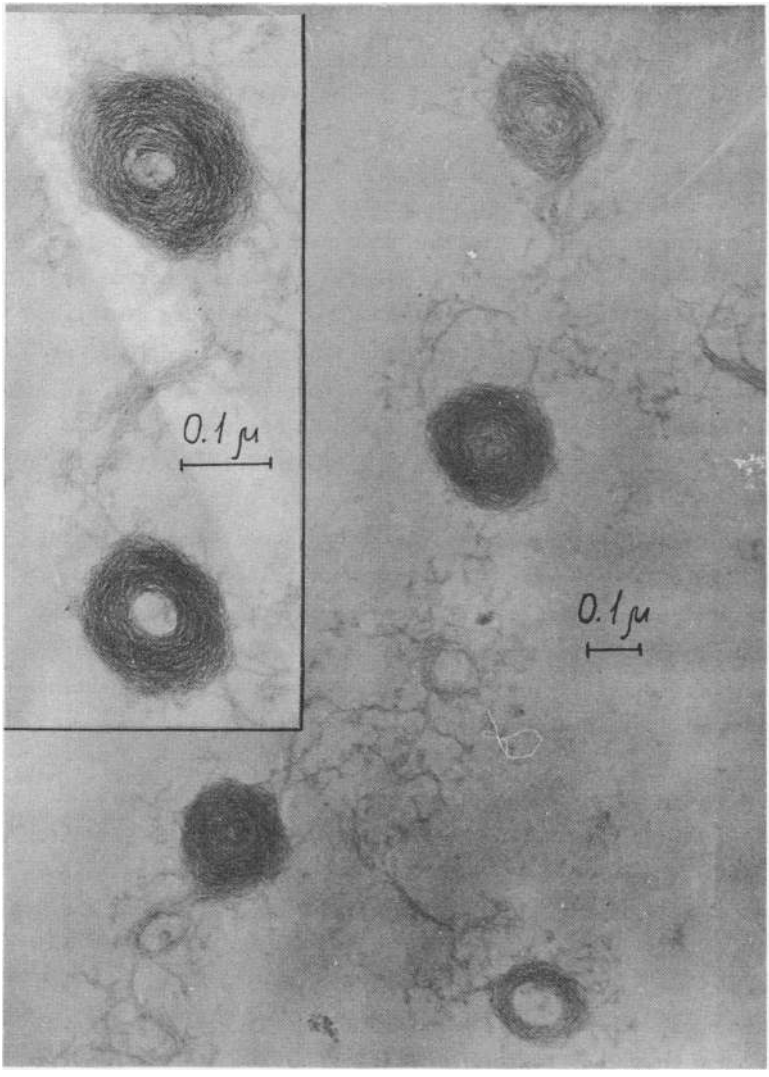


Fig.2. X-ray powder diffraction patterns of *E. coli* DNA compact particules, obtained by the procedure (I) at C_{PEG}=150 mg/ml; m.w.$_{PEG}$=20,000. a) A wide-angle pattern. b-d) Small-angle patterns, characteristic of different *E. coli* DNA preparations (in the case (d) the sample was partially oriented).

2d). Compact particules, formed from all other types of DNA (see Materials and Methods) showed an intense reflection I and a more weak and diffuse reflection II.

It was found out that in the case of any particular *E. coli* DNA preparation the small-angle pattern did not change with the molecular weights of DNA (1.3×10^7-5.4×10^5) and of PEG (4,000-20,000), while the value of d_I slightly varied with the PEG concentration (d_I=34-37 Å and ~40 Å at C_{PEG}= 150 and 80 mg/ml, respectively). A tenfold increase in the DNA concentration above the usually used value of about 20 μg/ml did not cause any noticable changes in small-angle patterns.

Fig.3a shows an electron micrograph of the DNA compact particules, formed by the procedure (I) at C_{DNA}~ 10 μg/ml and C_{PEG}=120 mg/ml. In accordance with results, obtained earlier[3,4], the particules appear as doughnuts (sometimes as discs) 1,500-2,500 Å in diameter, the diameter of the hole being 500±150 Å. Some structure appears to be discernible in these



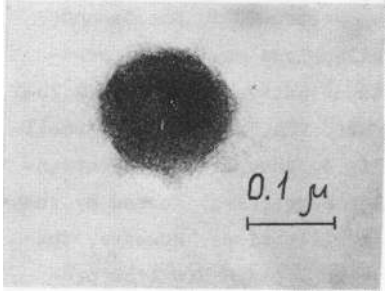


Fig.3. Electron micrographs of DNA compact particules, formed from: a) native DNA (by the procedure (I)); b) heat denaturated DNA (by the procedure (2)).
$C_{DNA}=10^{-6}$ g/ml; $C_{PEG}=120$ mg/ml; m.w.$_{DNA}=10^{6}$; m.w.$_{PEG}=20,000$

particules.

Compact particules, formed from DNA molecules with disordered secondary structure. Fig.4 shows the CD spectra of *E.coli* DNA, brought into a compact (or a "condensed") state according to the procedures (2), (4) and (5), together with the spectrum of a PEG-free DNA solution. It can be seen that the spectra of compact particules, formed from heat denaturated DNA (the procedure (2), curve 2), and of the DNA "condensed" form, obtained in the absence of PEG (the procedure (4), curve 3), resemble the spectrum of native DNA in a PEG-free solution (curve I), though with markedly diminished amplitudes of the positive and the negative bands. The spectrum of DNA, precipitated with ethanol (the procedure (5), curve 4) resembles that of DNA in the A form. The intensities of both bands in the CD spectra of the compact particules, formed from acid denaturated DNA (the procedures (3a,b)), were close to zero.

Thus all types of the DNA particules, described above, do not display the Ψ CD spectrum, which is characteristic of compact particules, formed from native DNA molecules (see Fig.I). It should be pointed out that in all these cases the DNA molecules were double-stranded, but in contrast to native DNA their secondary structure was more or less disordered. The heat denaturated DNA molecules, used according to the procedure (2), had some double-stranded regions due to their partial renaturation, as judged by the remaining hyperchromicity value of about 10%. The acid denaturated DNA molecules (the procedures (3a,b) and (4)) also must have some double-stranded regions, since a complete strand separation does not occur under the employed conditions[20]. The precipitation of DNA with ethanol also does not lead to the DNA strand separation.

Two examples of small-angle X-ray diffraction patterns of compact particules, formed from acid denaturated DNA by the procedure (3a) are presented in Fig.5. They show only the reflection II (d_2=84 Å) and its second-order 42 Å reflection. The comparison of the patterns, presented in Fig.5a and 5b, demonstrates, that the intensity of these reflections may vary depending upon the DNA preparation, similar to the case of native DNA (see Fig.2b-d). Compact particules, formed from heat denaturated DNA, produced the small-angle patterns, similar to those, presented in Fig.5, though the reflection II became more diffuse and less intensive. Compact particules, formed by the procedures (3a), (3b) and (4) generated identical reflections. However, the acidification of high molecular weight DNA (m.w.$\sim 10^7$) to pH 2.0 (the procedure (4)) resulted in a formation of macroscopic aggregates, which did

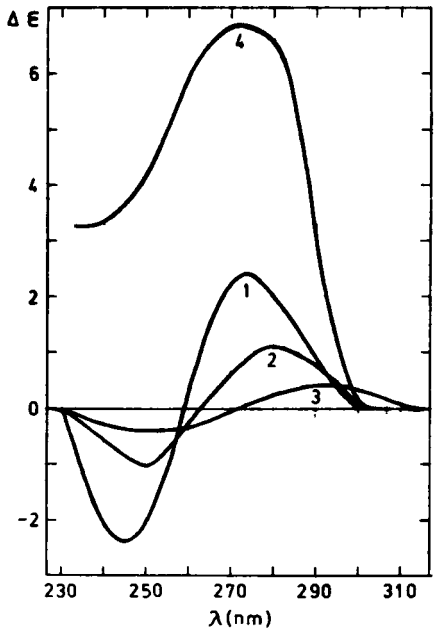


Fig.4. CD spectra of *E.coli* DNA molecules with partially disordered secondary structures in PEG-free and in PEG-containing solutions: I - in the absence of PEG; 2 - heat denaturated DNA (the procedure (2)), C_{PEG}=150mg/ml, m.w.$_{PEG}$=20,000, m.w.$_{DNA}$=10^7; 3 - DNA (m.w.=8.1x10^5), acidified to pH 2.0 (the procedure (4)); 4 - DNA (m.w.=8.1x10^5), precipitated with ethanol (the procedure (5)).

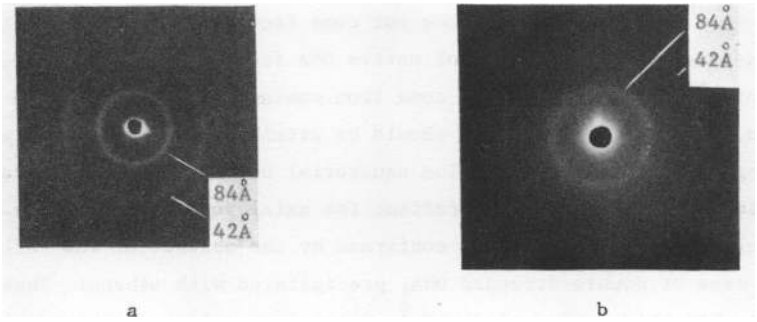


Fig.5. Small-angle X-ray diffraction patterns of compact particules, formed from acid denaturated *E.coli* DNA by the procedure (3a). C_{PEG}=150 mg/ml; m.w.$_{PEG}$=20,000; m.w.$_{DNA}$=8.1x10^5. a), b) - patterns, characteristic of different *E.coli* DNA preparations.

not generate any small-angle reflections. Neither were the small-angle reflections observed with ethanol precipitated DNA samples. Wide-angle X-ray

diffraction patterns of compact particules, formed from denaturated DNA, showed only one diffuse ring at a spacing of about 3.4 Å.

Fig.3b shows an electron micrograph of a typical particule, formed from heat denaturated DNA (the procedure (2)). It can be seen that such particules have the shape and dimensions almost the same as in the case of native DNA (see Fig.3a); however, they never possess a hole in the centre and a definite substructure, observed with native DNA.

The experimental results, described above, demonstrate that compact particules, formed in PEG-containing water-salt solutions from native DNA molecules under various conditions (different molecular weights of DNA and of PEG, different PEG concentrations) always produce a typical ψ CD spectrum and a small-angle X-ray diffraction pattern which shows two reflections: the reflection I at d_I=34-40 Å and the reflection II at d_2=80-90 Å. As for the particules, formed from DNA molecules with disordered (or altered) secondary structure, they show neither the ψ CD spectrum, nor the reflection I; their small-angle X-ray diffraction patterns contain only the reflection II.

Let us consider at first the reflection I, which is characteristic of compact particules, formed from native DNA. The data, described above, demonstrate that: I) the samples of compact particules, formed from heat or acid denaturated DNA did not show the reflection I; 2) the position of the reflection I was independent of the molecular weight of PEG. These data, togeter with the fact that small-angle X-ray diffraction from crystals or oriented films of PEG (m.w. 20,000) yielded only a diffuse ring at about I50 Å, indicate that the reflection I does not come from PEG. Since the reflection I was observed with the samples of native DNA from different sources, it may be inferred that it does not come from contaminations in the DNA preparations. Thus the reflection I should be attributed to native DNA molecules being in the compact form. The equatorial orientation of this reflection indicates that it does not reflect the axial periodicity of the DNA double helix. This conclusion is confirmed by the absence of the reflection I in the case of double-stranded DNA, precipitated with ethanol. Thus one may infer that the spacing of 34-40 Å arises from a long-range ordering of DNA chains in compact particules. Since the period of 34-40 Å is close to the dimensions of the unit cells, observed in "crystalline" DNA fibers[23], it is plausible to suppose that the reflection I is assosiated with a side-by-side packing of DNA fragments, which have been drawn together in the course of compactization and form ordered regions, which one may call "mic-

rocrystalline". Similar interpretation was given[15] to the low-angle X-ray scattering maximum, which was observed for DNA in PEG-containing solutions at high DNA concentrations ($C_{DNA} \sim 350$ μg/ml). This scattering maximum corresponds, most probably, to the reflection I under consideration, since it has been shown (see above) that the small-angle X-ray diffraction pattern of DNA compact particules was independent of the DNA concentration (in the range 20-250 μg/ml).

In accordance with the X-ray diffraction data, electron micrographs show that the compact particules, formed from native DNA molecules (see Fig.3a) possess not only a more or less uniform size and a specific doughnut shape, but also a definite internal structure. On the contrary, compact particules, formed from heat denaturated DNA, never possess a characteristic doughnut shape and lack any detectable substructure (see Fig.3b).

Thus, only native double-stranded DNA molecules, being driven into a compact state, form particules with an ordered internal structure, which is characterized by the period of 34-40 Å. It may be supposed that such ordering (the formation of "microcrystalline" regions) involves specific interactions between adjacent fragments of the DNA double helixes, which may be realized only in the case of a perfect secondary structure.

Another important conclusion may be driven from the comparison of the X-ray diffraction data and the CD spectra of the DNA compact form. Since aggregation of DNA is not always followed by the appearance of the Ψ spectrum, as it happens in the case of compact particules, prepared by the procedures (2)-(5), it may be inferred that the Ψ spectrum is not merely an optical artifact, produced by aggregation, but reflects, as suggested earlier[3,8,14] certain pecularities of the internal structure of the compact particules. It has been shown above that in the case of native DNA the reflection I and the Ψ spectrum always appear simultaneously. This correlation suggests that the Ψ spectrum is generated by an ordered (possibly side-by-side) arrangement of DNA double helixes within the compact particules. Data, available from the literature, give further support to this conclusion, as it has been shown[24,25] that a formation of a cholesteric liquid-crystalline structure may be accompanied by specific optical effects, in particular, by a CD spectrum with intense negative or positive bands.

As for the reflection II, the results, described above, show that it is essentially independent of whether the DNA molecules possess a native secondary structure or not. The fact that this reflection does not change with the molecular weight or with the concentration of PEG and appears also

in the case of the DNA "condensed" state, obtained in the absence of PEG (the procedure (4), sonicated DNA) indicates that the reflection II does not come from PEG. It is hard to imagine that the reflection II can come from impurities in the DNA preparations, since it was observed with DNA from six different sources and disappeared in the cases of intermolecular aggregation of DNA in ethanol or at pH 2.0 (the procedure (4), high molecular weight DNA). The data, available at present, do not provide enough information for a reliable interpretation of the reflection II. However, it should be noted that the spacing of 80-90 Å does not contradict, for example, to a lateral packing of DNA superhelixes (according to different models, the external diameter of the DNA superhelix may vary from 40 to 106 Å[26-29]) or to a "folded-chain" structure[30-32]. Similar reflections were observed (though not discussed) in concentrated gels of nucleohistones[33]. The equatorial spacing of 80-90 Å may be assosiated also with the possible additional ordering of the compact particules during the centrifugation step and correspond, for example, to the "thickness" of the particule. In either case the regularity, which accounts for the reflection II, does not generate the specific Ψ CD spectrum of compact particules.

As mentioned above, the wide-angle X-ray diffraction pattern or DNA compact particules, in accordance with results, obtained earlier[15], shows a set of reflections, which resemble those, characteristic of DNA in the B-fiber structure. However, this fact provides no basis for the conclusion that the whole DNA molecule within the compact particule is in the B form. For example, from the model of ordered compact particules, proposed above, it follows that the confermation of some DNA fragments, which interact with each other within the "microcrystalline" regions, may differ from that of the B form, while other DNA fragments will retain the B conformation and give the corresponding wide-angle pattern.

The above-made comparison of diffraction and CD patterns of DNA compact particules, formed under various conditions, reveals essential differences in their internal structure. It was found out that compactization of DNA is not necessarily accompanied by a formation of "microcrystalline" regions in compact particules and, as a consiquence, by the generation of the Ψ CD spectrum. In this connection the use of the term "Ψ-form" as acronym of "polymer-salt-induced" (psi) for description of all cases of DNA compactization in the presence of salt and polymer does not seem to be justified. We suppose the use of this term to be expedient for designation of those compact particules, which were formed from native DNA molecules and

produce a ψ CD spectrum and an X-ray diffraction pattern, indicative of an ordered internal structure of the particules.

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*) Abbreviations used are: PEG - poly(ethylene glycol); CD - circular dichroism.

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