A COMPACT FORM OF DOUBLE-STRANDED DNA IN SOLUTION

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1. Introduction

The conformational transition of DNA during its functioning *in vivo* does not terminate in the entire separation of strands and in the formation of single-stranded coils as it occurs in the case of the thoroughly studied *in vitro* conformational "helix-coil" transition. Comparing various structural states of double-stranded DNA molecules in chromosomes of viruses, bacteria and eukaryotes, one may conclude that under the specific impact of certain unknown agents the relative "rigidity" of helices of DNA *in vivo* may alter and they may form compact structures without separation of strands.

We may suppose that in order to bring about such kind of conformational transition of DNA *in vitro* it is necessary, first of all, to eliminate the reasons which cause the "rigidity" of double-stranded helices (specific properties of the hydration shell, the charges of the phosphate groups, etc.) without creating the extreme conditions which facilitate the separation of strands (pH, temperature).

In the present study an attempt has been made to obtain compact double-stranded molecules of DNA in solution and to determine some physico-chemical properties of the ensuing structural form of DNA. Taking into account the capability of a number of "neutral" polymers to compete with DNA for water molecules it might be hoped that under the influence of such polymers (in particular of polyethyleneglycol) the structural state of the hydration shell of DNA would alter and the relative "rigidity" of the double-stranded helices would be decreased. If the concentration of counter-ions screening the charged phosphate groups of DNA be simultaneously increased, it would be reasonable to expect the conditions favourable for conformational transition of the double-stranded helices of DNA in solution without strand separation. Proceeding from the above considerations we have studied the structural state of DNA in water–salt solutions in presence of PEG.

Abbreviations: PEG, polyethyleneglycol; C_{DNA} and C_{PEG}, concentrations of DNA and PEG, respectively, circular dichroism.

2. Materials and methods

E. coli DNA ($s_{20,w}$ = 25; M_w = 14 × 10^6; E_p = 6780) and phage SD DNA ($s_{20,w}$ = 27; M_w = 16 × 10^6; E_p = 7150) were isolated and purified by standard procedures. Chicken erythrocyte DNA ($s_{20,w}$ = 10.6; M_w = 1.2 × 10^6; E_p = 6700) was purchased from "Reanal" (Budapest). T7 DNA, actino-phage DNA and *B. subtilis* DNA were kindly given to us by Dr. E. Sverdlov, Dr. V. Permogorov and Dr. A. Krivisky. Crystalline PEG (M_w = 20,000) was purchased from "Schuchardt" (München). The water–salt solutions of PEG were prepared by dissolving certain amounts of PEG in NaCl solutions of required molarity.

Electron micrographs were taken with JEM-7 at 50,000 screen magnification. A droplet of the DNA–PEG mixture being investigated was deposited on the parlodion-coated grid, stained for 5 min with 1% uranyl acetate in 70% acetone and dried on filter paper. The absorption spectra were recorded with a "Specord"-UV-vis (Zeiss, Jena) and the CD-spectra with a "Roussel-Jouan"-CD-180 dichrograph. C_{DNA} in optical measurements did not exceed 50 μg/ml. The acidification of PEG-containing DNA solutions was performed by gradual addition of certain volumes

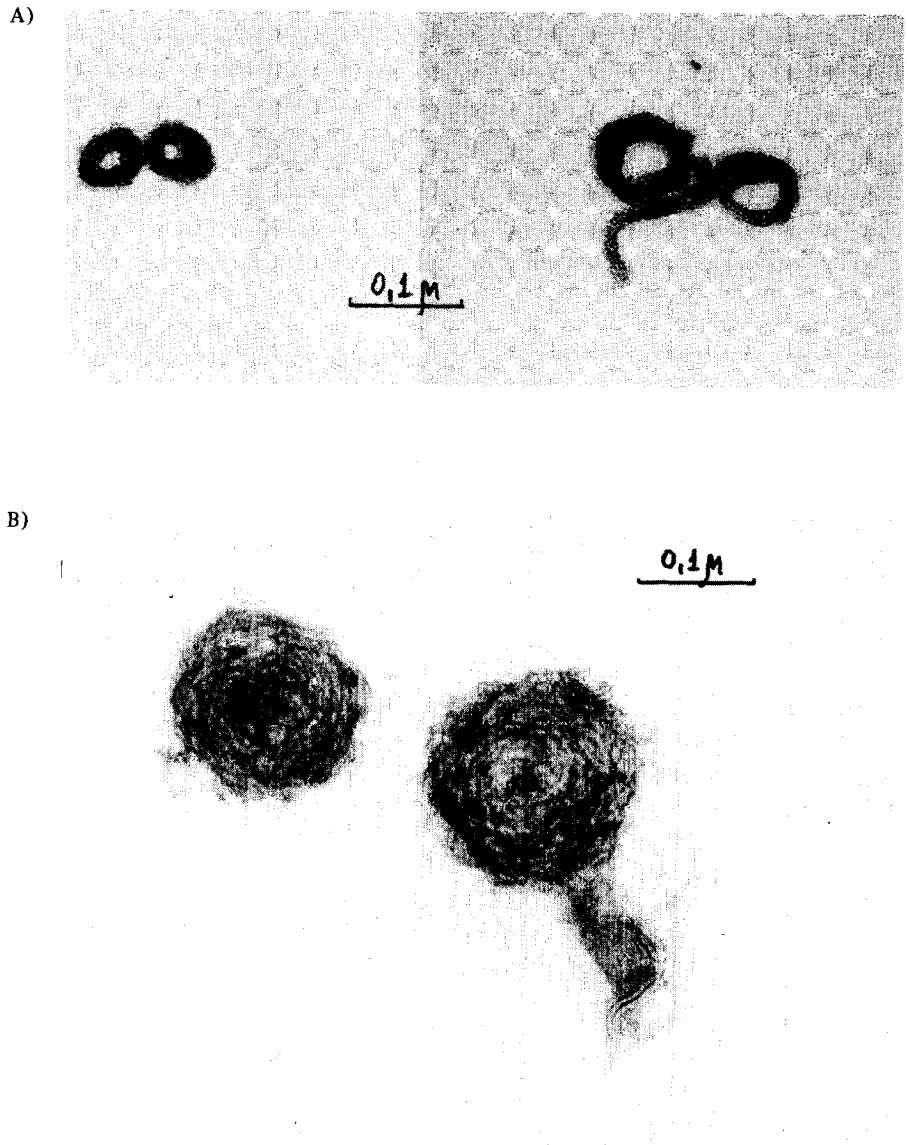

Fig. 1. Electron micrograph (× 150,000) of structural forms of *E. coli* DNA at various concentrations of PEG. Initial solution: $C_{DNA} \simeq 50$ μg/ml, 0.25 M NaCl. A) Doughnut-shaped form (C_{PEG} = 80 mg/ml); B) Less compact ("globular") form (C_{PEG}= 60 mg/ml).

.of 0.1 N HCl to the initial solutions (2 ml). As the volume of added HCl solution never exceeded 50–60 μl, the corrections for dilution were not introduced.

3. Results and discussion

Fig. 1 shows the electron micrographs of structural forms of DNA in PEG-containing water–salt solutions at various PEG-concentrations. At C_{PEG} = 80 mg/ml the DNA molecules have a compact hollow doughnut-shaped form (fig. 1A); when C_{PEG} is lower than 80 mg/ml the structural form of DNA is less compact (fig. 1B). The electron micrographs of DNA at C_{PEG} about 50 mg/ml and lower enable one to observe the early stages of formation of the DNA compact structure. It should be noted that DNA in the form of hollow doughnut particles was observed by M. Haynes et al. [1] and by D. Olins and A. Olins [2] in their studies of complexes of polyamino acids and histones with nucleic acids.

Fig. 2 shows, as an example, the absorption spectra of *E. coli* DNA solutions (C ≃ 30 μg/ml) measured at various molar ratios of PEG/DNA. One can see that at C_{PEG} exceeding a certain critical value, the absorption curves of DNA substantially differ from that of PEG-free solution, the intensity of the band at 260 nm decreases with increasing C_{PEG}. The similar dependence of the shape of the spectra upon C_{PEG} have been observed for other DNA preparations. The comparison of DNA absorption spectra at different ionic strengths shows that the critical concentration of PEG decreases with the increase of ionic strength. The comparison of the observed effects for various C_{DNA} and for DNA preparations from different sources permits one to reach a preliminary conclusion that the critical C_{PEG} value depends not only on the C_{DNA} but also on the content of G–C base pairs and on the M_w of DNA.

Fig. 3 shows the effect of PEG upon the *E. coli* DNA absorption spectrum (C_{DNA} ≃ 30 μg/ml) on acidification of the solution. It is easy to observe that acidic titration of DNA in the presence of PEG (fig. 3B) is not accompanied by a hyperchromic effect which characterizes the acid denaturation of DNA in PEG-free solution (fig. 3A). Thus, the compact form of double-stranded DNA in PEG-containing solutions differs from the normal double-stranded helices not only by the shape of their absorption spectra but also by their ability to undergo acid denaturation.

Fig. 4 shows, as an example, the typical CD curves of *E. coli* DNA solutions (C_{DNA} ≃ 10 μg/ml) measured at various C_{PEG}. As may be seen, the shape of the curve is altered with the increase of C_{PEG}. The

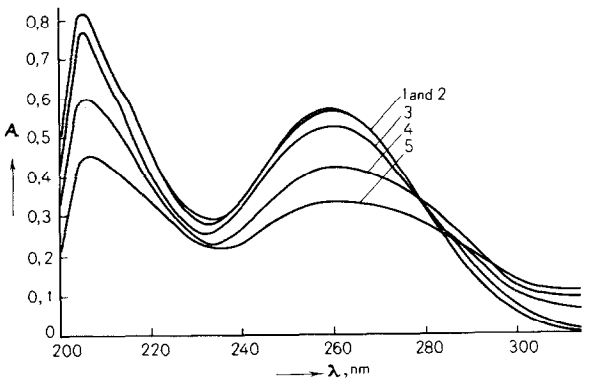

Fig. 2. Dependence of the shape of UV-spectrum of *E. coli* DNA upon the concentration of PEG (C_{DNA} ≃ 30 μg/ml; 0.25 M NaCl). 1) C_{PEG} = 0; 2) C_{PEG} = 40 mg/ml; 3) C_{PEG} = 60 mg/ml; 4) C_{PEG} = 80 mg/ml; 5) C_{PEG} = 100 mg/ml.

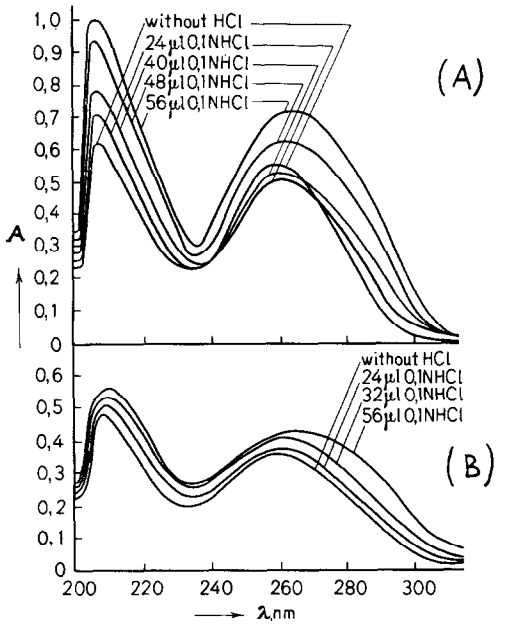

Fig. 3. The effect of PEG upon DNA absorption spectra at acidic titration by 0.1 N HCl (C ≃ 30 μg/ml; 0.25 M NaCl) (for the procedure of titration see text). (A) C_{PEG} = 0; (B) C_{PEG} = 60 mg/ml.

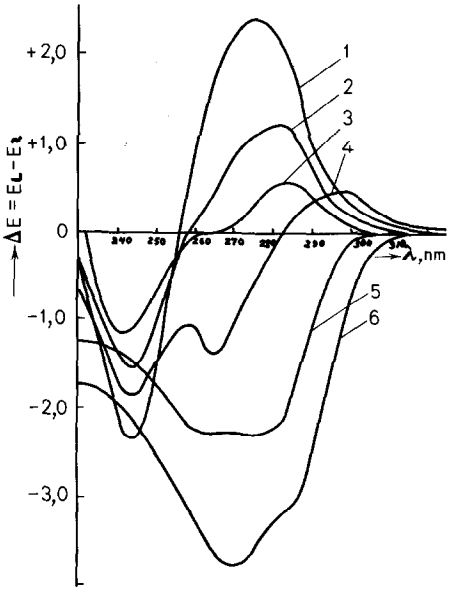

Fig. 4. Dependence of the shape of the CD spectrum of *E. coli* DNA upon the C_{PEG} ($C_{DNA} \simeq 10$ μg/ml; 0.25 M NaCl). 1) $C_{PEG} = 0$; 2) $C_{PEG} = 40$ mg/ml; 3) $C_{PEG} = 80$ mg/ml; 4) $C_{PEG} = 100$ mg/ml; 5) $C_{PEG} = 120$ mg/ml; 6) $C_{PEG} = 140$ mg/ml.

increase of C_{PEG} at low values of C_{PEG} (0–80 mg/ml) is accompanied by a gradual decrease of amplitude of both positive and negative bands as well as by a red shift of the positive band maximum. At higher values of C_{PEG} (80–140 μg/ml), the positive band gradually disappears and a new intense negative band with a maximum about 270 nm appears. The CD spectra measured at different ionic strengths demonstrate that, as is the case with UV-absorption spectra, the C_{PEG} value, at which the negative band shows up, depends largely upon the ionic strength; the greater the ionic strength the lower the "critical" value of C_{PEG}. The changes in the shape of CD spectrum of diluted DNA solution ($C_{DNA} = 10$ μg/ml) with a low C_{PEG} (10–60 mg/ml) brought about in the process of its acidification do not differ substantially from those occurring upon acidification of PEG-free DNA solutions; in both cases not only the amplitudes of the positive and negative bands are decreased but a new band at 257–258 nm (which is characteristic of the protonated state of G–C base pairs) is seen to appear. If, however, C_{PEG} is over 60–80 mg/ml the acidification of DNA solution is accompanied by a sharp decrease of the amplitude of the negative band. These data testify to the fact that, in contrast to the main band in the absorption spectrum (see fig. 3), the negative band in CD spectrum is a sensitive parameter of protonation of the compact form of DNA.

The considerable differences in the shape of CD spectra of DNA at PEG-contents lower and higher than the critical one, have enabled us to estimate the extent of reversibility of the DNA structural transition under study. The comparison of the CD spectra of PEG-containing DNA solution ($C_{DNA} = 10$ μg/ml; $C_{PEG} = 90$ mg/ml) and the solution obtained after its 2-fold dilution with water, evidences to reversibility of the transition. It should be pointed out, however, that within 1–2 hr no complete restoration of the spectrum is observed; this may be explained by a low rate of restoration of the structure or by a partial irreversibility of such a structural transition. Experiments with *B. subtilis* DNA have shown that compactization of DNA with subsequent restoration of the initial structure by dilution of the PEG-containing DNA solution does not result in the loss of transforming activity of this DNA. This also suggests reversibility of the observed structural transition of DNA.

It must be noted that when the present investigation was in progress, L.S. Lerman published an article [3] reporting an increase of DNA sedimentation rate in water–salt solutions upon addition of poly(vinylpyrrolidinone) or of poly(ethylene oxide) which was attributed to the formation of a compact DNA structure. It is essential that the sedimentation coefficients of DNA do not depend, according to Lerman, in DNA concentration and this provides strong evidence for the intramolecular nature of the conformational transition.

The data presented in this paper show that addition of PEG to a water–salt solution of DNA results in decrease of relative "rigidity" of the double-stranded helices and in conformational transition of DNA which lead to a compactization of its structure. It was found that this transition occurs more readily the higher the ionic strength of solution and the concentration of PEG. At present studies are in progress on the heat effects of mixing water–salt solutions of DNA and PEG and on some hydrodynamic properties of DNA in PEG-containing solutions.

It may be thought that both by its nature and by the configuration of the compact form of DNA, such

transition is similar to the conformational transitions of DNA during its functioning in the living cell.

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References

[1] M. Haynes, R.A. Garret and W.B. Gratzer, Biochemistry 9 (1970) 4410.
[2] D.E. Olins and A.L. Olins, J. Mol. Biol. 57 (1971) 437.
[3] L.S. Lerman, Proc. Natl. Acad. Sci. U.S. 8 (1971) 1886.