VISCOSIMETRIC STUDY ON COMPACT FORM OF DNA IN WATER–SALT SOLUTIONS CONTAINING POLYETHYLENEGLYCOL

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1. Introduction

It has been shown [1,2] that the shape of the absorption and CD spectra of DNA in water–salt (WS) solutions are altered by the addition of polyethyleneglycol (PEG). These alterations, particularly the increase in optical density at the wavelengths exceeding 320 nm, the decrease of the band intensity at 260 nm in the absorption spectrum, and the appearance of a new intense negative band in the CD spectrum observed in PEG-containing WS-solutions of native DNA at room temperature, resemble the alterations occuring in native DNA spectra after adding certain polycations such as, for example, polyamino acids [3–5].

According to the available data [6], the observed alterations in the optical properties of DNA after addition of PEG may not be accounted for by DNA denaturation. These data made it possible to draw the conclusion that the double-stranded DNA molecules in PEG-containing WS-solutions tend to form compact particles visualizable by means of the electron microscope [6]. Data on the rate of DNA sedimentation indicating the formation of compact DNA particles in the presence of PEG and some other polymers have obtained by Lerman [7].

In the present study the influence of PEG upon the structural state of DNA was investigated by means of viscosimetry.

Abbreviations: C_{DNA} and C_{PEG} concentrations of DNA and PEG, respectively.

2. Materials and methods

Phage SD DNA isolated by the procedure described in [8] was used. The molecular weight of DNA as estimated by its intrinsic viscosity in 0.25 M NaCl [9], was 20×10^6. The DNA concentration of the solutions used in these experiments was 20 μg/ml.

The PEG preparations from 'Schuchardt' (GFR) (mol. wt. = 400 and 20 000) and from 'Ferak' (West Berlin) (mol. wt.= 40 000) were used without additional purification. The required PEG concentration in WS-solutions was achieved by mixing corresponding WS-solutions of DNA and PEG in 0.25 M NaCl.

The relative viscosity of WS-solutions of DNA with a fixed PEG concentration was calculated with respect to WS-solutions containing PEG of the same concentration. The measurements of viscosity were made by a magnetic rotation viscosimeter [10], the principle of which was suggested by Zimm and Crothers [11].

3. Results and discussion

The dependence of specific viscosity on concentration of PEG of different molecular weight, is shown in fig. 1. The diagram indicates that within a certain range of PEG concentrations the specific viscosity of DNA is nearly the same as in the PEG-free WS-solution. The range of these concentrations depends upon the molecular weight of PEG. Thus, for instance, in the case of PEG with mol. wt.=400, the specific

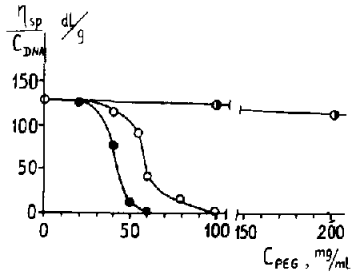

Fig. 1. The dependence of the value η sp/C_{DNA} upon C_{PEG}; (⊙) mol. wt. PEG = 400, (○) mol. wt. PEG = 20 000, (●) mol. wt. PEG = 40 000.

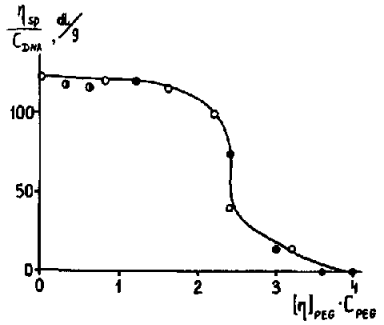

Fig. 2. The dependence of the value η sp/C_{DNA} upon the product $[\eta]_{PEG} \times C_{PEG}$; (⊙) mol. wt. PEG = 400, (○) mol. wt. PEG = 20 000, (●) mol. wt PEG = 40 000.

viscosity of DNA remains practically constant up to C_{PEG} = 200 mg/ml. The negligible decrease of the value η sp/C_{DNA} at high concentrations of PEG, observed in this particular case, may be explained by some worsening of the property of the solvent for DNA. If, however, we use PEG preparations with molecular weights 20 000 and 40 000, the value η sp/C_{DNA} remains constant till C_{PEG} = 20–40 mg/ml. Further increases of PEG concentration are accompanied by a sharp decrease of the value η sp/C_{DNA}. If C_{PEG} reaches the value of about 60 and 100 mg/ml as in the case of PEG preparations with the molecular weight 40 000 and 20 000 respectively; the contribution of DNA (at concentration 20×10^{-6} g/ml) to the viscosity of PEG-containing solutions becomes negligibly small. It should be pointed out that within the range of the decrease of the value η sp/C_{DNA}, the relative viscosity of DNA alters with the time, and the data obtained correspond to the equilibrium values of η sp/C_{DNA}.

The pattern of dependence of the value η sp/C_{DNA} upon C_{PEG} demonstrates that within a certain range of PEG concentrations the DNA molecules undergo a conformational transition which results in the formation of a compact structrue. the extent of cooperativity of such a transition depends in some measure upon the mol. wt. of PEG.

To characterize the conformational transition of DNA molecules quantitavely it is necessary to determine the value $[\eta]_{DNA} = \lim (\eta \text{ sp}/C_{DNA})\ C_{DNA} \to 0$ proceeding from values η sp/ C_{DNA} measured over a wide range of DNA concentrations at different values of C_{PEG}. However, the tendency of DNA to aggregate at high values of C_{PEG} precludes such measurements. Therefore, the data shown in fig. 1, which show a considerable collapse of the DNA molecules in PEG-containing solutions are of but a qualitative character.

The observed conformational transition of DNA is to a considerable extent reversible. This is confirmed by the fact that the relative viscosity of WS-solutions of DNA in the presence of low concentrations of PEG (in which the compact particles of DNA do not form) do not differ appreciably from the relative viscosity of similar solution obtained by diluting high PEG-concentration solutions with WS-solvent (taking into account the decrease of DNA concentration). This fact enables us to suppose that no stable bonds between DNA and PEG molecules occur and that the observed viscosimetrical properties of DNA molecules in the presence of PEG are brought about by the limit of the volume for this macromolecule in the solution. Since the values $[\eta]_{PEG}$ for the preparations of PEG used are well known, we may multiply $[\eta]_{PEG}$ by C_{PEG}, and this product may characterize the part of the volume occupied by PEG molecules in the solution at the indicated values of C_{PEG}. Fig. 2 shows the dependence of the value η sp/C_{DNA} upon the product $[\eta]_{PEG} \times C_{PEG}$; this dependence is described in a satisfactory fashion by one and the same curve for the preparations of PEG with mol. wt. 400, 20 000 and 40 000. A certain scattering of the experimental points at high values of C_{PEG} is due to errors in measurements because in this range of C_{PEG} the relative viscosities of DNA are extremely small.

The data shown in fig. 2, indicate a possible relation between the volume occupied by PEG molecules in solution and the conformational transition of DNA. This provides a possible explanation of the behaviour

of DNA molecules in solution containing PEG with mol. wt. = 400. In this case, even at high PEG concentrations (100 and 200 mg/ml), the values of $[\eta]_{PEG} \times C_{PEG}$ remain small (fig. 2). A small deviation of the points corresponding to the PEG preparation with low molecular weight, is connected, as was previously pointed out, with the worsening of the solvent for DNA at high PEG concentrations.

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