Conformation of Proflavine-Bound DNA Molecules

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The conformational changes suffered by full-length double-stranded DNA molecules from bacteriophage T7, due to their binding with the mutagenic dye proflavine (PF), has been studied by electron microscopy. The dye-bound DNA showed a smoother contour and a more compact configuration in comparison with the dye-free native DNA. These changes resulted from the stretching, extension and charge-neutralization accompanying the incorporation of PF.

Introduction

The extension of the DNA molecule, due to its binding with the mutagenic drug proflavine (PF) has been established by direct measurement of the contour length of the dye-DNA complexes [1, 2]. However, the evidences regarding the alterations of flexibility caused by the binding, are rather uncertain. The dichroic properties of the DNA and those of the bound acridines have been interpreted to show a stiffening of the macromolecule [3]. This conclusion has been supported by a later electron microscopic study on DNA-ethidium bromide complex [4]. The hydrodynamic and electro-optical properties of DNA-acridine complexes, on the other hand, indicate that the binding renders the DNA double helix more flexible than its native, dye-free state [5–7]. In view of these contradictory results, an investigation on the conformation of the dye-bound DNA was considered worthwhile. Since electron micrographs of DNA furnished a visual representation of the instantaneous configuration of the macromolecule in solution [8], an electron microscopic comparison between the spatial organization of the free and PF-bound DNA was undertaken in the present study.

Materials and Methods

Neutral proflavine hemisulphate (PF) was a gift from Imperial Chemicals, Manchester, U.K. The dye was dissolved in 0.5 M NaCl and stored in dark at 4 °C.

E. coli virus T7, was cultured from a stock originally supplied by Dr. A. Guha, Toronto University, Canada and was purified by differential centrifugation as described earlier [9]. The isolated bacteriophage was suspended in 0.5 M NaCl and the DNA was extracted by heating it at 65 °C for 20 min.

The molar concentration of the dye and the DNA were determined spectrophotometrically assuming the extinction coefficients at 440 and 260 nm to be 41,000 M^{-1} cm^{-1} and 6,600 M^{-1} cm^{-1} respectively.

DNA-PF complexes were prepared at DNA phosphate (P) to dye (D) ratio of 1.0 where almost all of the binding sites might be considered to be occupied [2, 10]. The complex solution in 0.5 M NaCl was mixed with 0.04% cytochrome c (Nutritional Biochemicals, U.S.A.) in the same solvent and the mixture was spread on a cleaned surface of triple-distilled water, containing PF at the same concentration as in the DNA-dye complex solution. The DNA molecules, adsorbed to the protein monolayer, were picked up on carbon-coated copper grids, rotary shadowed, and micrographed by a Siemens Elmiskop I operating at 40 kV as already described [2].

Results and Discussion

A few typical full length native DNA molecules from T7 spread on a salt-free hypophase are shown in Fig. 1 *. All the molecules exhibited wide circular loops. The contours were smooth and less kinky than preparations on salt-hypophase which indicated considerable stretching of the polymer due to intramolecular electrostatic repulsion in absence of

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* Figs 1 and 2 see Table on page 130 a, b.

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counterions in the bulk solution[9]. The wide bends demonstrated the inherent structural rigidity of the double stranded DNA.

Fig. 2 represents the T7 DNA molecules at P/D = 1. The configurational pattern had remarkably changed. The loops lost their circularity and were bounded by straight regions with comparatively sharp bends. The dye-bound macromolecules were more compact with numerous foldings on themselves and occupied smaller spatial domains in comparison to the free state in spite of the 27% extension (from 13.13 nm to 16.75 nm)[2]. The contour characteristic was distinctly smoother and completely kink-free.

The increased smoothness and the straightening of the contours in the PF-bound DNA suggested a stretching and stiffening of the polymer which was consistent with the intercalation model. The unwinding[11] or winding[12] of the double helix, accompanying intercalation, caused a local stretching in the neighbourhood of the binding sites. This made the nucleotides of the dye-bound DNA more rotationally fixed in comparison to those in the dye-free DNA, and resulted in the kink-free, smooth appearance of the DNA in bound state.

However, the sharp bends and the increased compactness of the DNA-configuration strongly favoured the belief that the binding of PF increased the flexibility of the double helix. The reason for this rise in flexibility was less obvious at first sight. It might be pointed out that intercalation of sufficient number of PF molecules resulted in a considerable elongation of the DNA (27% increase in length was recorded at P/D = 1[2]). According to the hydrodynamic model of DNA, this increase in length added to the flexibility of the polymer[13, 14].

The electrostatic effects of the binding process were extremely important in the modification of the DNA configuration. The charge of the phosphate groups influenced the rate and extent of intercalation considerably[15, 16]. Reciprocally, it might be suggested that the intercalated cations influenced the electrostatic charge of the polymer. Any shielding of the anionic phosphates by intercalated PF cations would decrease the intramolecular coulombic repulsion and increase the flexibility of the DNA. Such neutralization of the phosphate groups by incorporation of PF was postulated by Claverie and Gilbert[17] and was verified from dielectric measurements on DNA-PF complex by Goswami, Das and Dasgupta[18].

In addition to intercalation, the binding process comprises a tangential attachment of the dye to the surface of the DNA double helix which is essentially electrostatic in nature. At P/D = 1, a large fraction of the dye is bound in this manner which reduces the surface charge without inducing any stretching of the polymer. The flexibility is considerably enhanced due to the decreased charge-charge repulsion among the various segments of the polyelectrolyte. This is manifested by the folded compact configuration of the dye-bound DNA. The consequences of stretching is reflected only in the smooth contour. These considerations are consistent with the optical and hydrodynamic characteristics of DNA-PF complexes. While the stretched contour and the specific orientation, resulting from intercalation, give rise to the enhanced dichroic ratio in bound state, the compact spatial domain accounts for the greater flexibility of the acridine-bound DNA observed in hydrodynamic measurements.

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