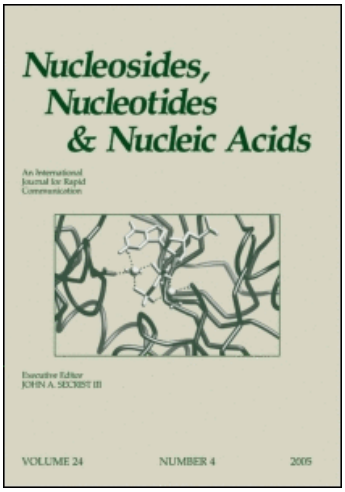

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Double-Stranded Nucleic Acids in Liquid-Crystalline Dispersions as Building Blocks for Cross-Linked Supramolecular Structures

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DOUBLE-STRANDED NUCLEIC ACIDS IN LIQUID-CRYSTALLINE DISPERSIONS AS BUILDING BLOCKS FOR CROSS-LINKED SUPRAMOLECULAR STRUCTURES

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ABSTRACT. Double-stranded DNA fixed in a cholesteric liquid-crystalline dispersion was used for generating an ordered supramolecular structure in the presence of anthracycline and copper (II) ions. The structure is stable in a water-salt solution and does not require poly(ethyleneglycol).The ordered network can be immobilized on the surface of a polymeric film, and may collapse in the presence of biologically and pharmacologically relevant compounds. Accordingly, the DNA-based liquid-crystalline network represents the basis to obtain novel highly sensitive biosensing units

INTRODUCTION

Biopolymers (nucleic acids, proteins) attract much attention as "building blocks" for innovative materials[1-3]. The application of synthetic or natural nucleic acids in this field is based on the wide spectrum of chemical and physical properties exhibited by these biopolymers. First, the intrinsic molecular "recognition" elements of nitrogen bases, inducing the self-assembling of nucleic acids, allows to build complex molecular structures or to functionalize pre-formed structures. Second, it is possible to modulate the properties of the above structures not only by changing their nitrogen base sequence, but also by modifying the properties of the solvent in which they are formed. Third, structures arising from biopolymeric molecules and inorganic current-conducting components as additional building elements would be relevant not only to the field of nanoelectronics, but also in terms of obtaining sensing units for analytical devices (biosensors)[4-5]. Therefore, the creation of networks in which molecules of biopolymers are

connected by inorganic elements, which can ensure both molecular "recognition" and possibility to transduce a signal, represents a goal of wide of interest.

We witness now an extensive demand of materials to be used for supramolecular design, but also of novel experimental approaches to obtain this type of structures. In particular, double-stranded nucleic acids (ds DNA), forming lyotropic liquid crystals, are presently used for supramolecular design[5-6].

We will discuss here the properties of ordered supramolecular networks based on double-stranded nucleic acids in the liquid-crystalline state[5-6].

MATERIALS AND METHODS

Double-stranded DNA from chicken blood erythrocytes (Reanal, Hungary) was used . The molecular mass of DNA after ultrasonic depolymerization, estimated by gel electrophoresis in 1 % agarose gel was 0.5-0.8 x 10^6 Da. The anthracycline daunomycin(DAU) was purchased from Sigma Chem. Co, USA. Poly(ethyleneglycol), PEG, (Serva, mol.wt. 4000 Da) was used. The concentration of DAU in water-salt solutions was determined spectophotometrically.

The solutions of DNA, DAU, PEG, NaCl were prepared on 2 mM Na-phosphate buffer (pH 6.67). The supramolecular structures of DNA were obtained as previously described[5] . Initially, the liquid-crystalline dispersion (LCD) of DNA was formed by mixing equal volumes of water-salt solutions (0.3 M NaCl; 2 mM Na-phosphate buffer; pH 6.67) of DNA and PEG. Subsequently, to 2 ml of LCD of DNA small (1-10 μL) volumes of DAU mother solution ($C_t \approx 4x10^{-3}$ M) were added under vigorous stirring. At the third stage, adjacent DNA molecules were "cross-linked" by polymeric chelate bridges, by addition of small (5-15 μL) volumes of $CuCl_2$ solution ($C_t = 1x10^{-3}$ M) to 2 ml of LCD of [DNA -DAU] complex under stirring.

CD spectra were recorded using a Jobin-Yvon, Mark III dichrograph (France), and the absorption spectra on a Specord - M 40 spectrophotometer (Germany). To isolate supramolecular structures, the solution in which they were formed was filtered through a poly(ethyleneterephtalate) nuclear membrane filter (diameter of pores 0.1-0.25 μM), that allowed to immobilize DNA particles; filters were dried on air not less than 1 hour. A surface of the nuclear membrane filter with immobilized DNA particles was examined by a SOLVER -P47 scanning atomic force microscope (NT- MDT, Zelenograd). The resonant mode was f = 546.4 kHz, the scanning area 13x14 microns, 512 x 512 pixels and the scanning rate 280 nm/sec.

RESULTS AND DISCUSSION.

The cholesteric packing the DNA is characteristic of conditions[7], at which the average distance between DNA molecules exceeds 30 Å . This causes the appearance of abnormal optical activity, i.e. an intense CD band in the absorption region of DNA chromophores (Fig.1 A, right panel). Curve 1, specific for a linear B-form of double- stranded DNA, is eventually transformed into curve 2, specific for a liquid-crystalline structure.

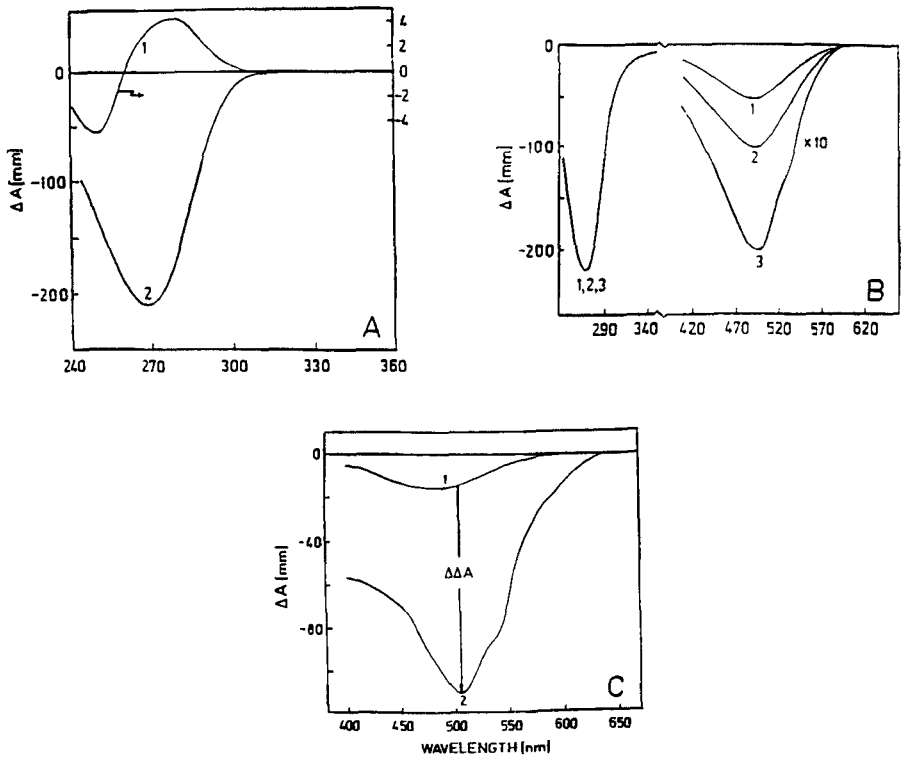

FIG. 1.

A. The CD spectra of linear double-stranded B-form of DNA (curve 1) and the DNA liquid-crystalline dispersion formed as a result of phase separation in water-salt-polymeric solution (curve 2).

Curve 1 – right ordinate; C_{DNA} = 5.2 mg/ml; 0.3 M NaCl + 0.002 M sodium phosphate buffer, pH 6.67;

Curve 2 – left ordinate; C_{DNA} =5.2 mg/ml; C_{PEG} = 170 mg/ml; 0.3 NaCl + 0.002 M sodium phosphate buffer; pH 6.67;

B. The CD spectra of the liquid-crystalline dispersions formed from (DNA – DAU) complexes.

Curve 1 – C_{tot} DAU = 4.1×10^{-6} M; curve 2 - C_{tot} DAU = 8.2×10^{-6} M; curve 3 - C_{tot} DAU = 16.4×10^{-6} M; C_{DNA} =5.2 mg/ml; C_{PEG} = 170 mg/ml; 0.3 NaCl + 0.002 M sodium phosphate buffer; pH 6.67;

C. The CD spectra of the DNA liquid-crystalline dispersions treated with DAU without (curve 1) and with (curve 2) added $CuCl_2$.

$C_{tot\ DAU} = 16.4 \times 10^{-6}$ M; $C_{tot\ CuCl_2} = 4.97 \times 10^{-6}$ M; C_{DNA} = 5.2 mg/ml;

C_{PEG} = 170 mg/ml; 0.3 NaCl + 0.002 M sodium phosphate buffer; pH 6.67.

Optical path - 1 cm; the dichroic absorption Δ A (A_L - A_R) and its change ΔΔA are given in mm; 1 mm = 10^{-5} optical units.

The formation of the liquid-crystalline dispersions (LCD) is not accompanied by reactivity changes of the nitrogen bases, yet the neighboring nucleic acid molecules in the new structure represent "building blocks" capable of chemical reactions. These can be used to generate supramolecular constructions with tailored properties. Indeed, the addition of anthracycline antibiotics to the liquid-crystalline DNA dispersion (Fig. 1 B, left panel) is accompanied by formation of (DNA-antibiotic) complexes, i.e. incorporation of antibiotic molecules. This results in the appearance of an additional intense (abnormal) band in the CD spectrum [8], located in the antibiotic absorption region (Fig.1B). The amplitude of the abnormal band is unequivocally determined by the amount of antibiotic bound to DNA (curves 1-3).

All studied anthracycline antibiotics[9] form complexes with DNA, the latter being easily detected by the appearance of the corresponding bands in the CD spectrum. The sign of the band in the antibiotic region is the same as in the DNA nitrogen base absorption region. This testifies that the orientation of antibiotic molecules, with respect to the long DNA helical axis, coincides with the orientation of the nitrogen bases.

Subsequent treatment of the dispersion of (DNA-antibiotic) complex with copper salts (Cu II ions) (Fig. 1 C) results in a manifold increase[10] in the amplitude of the band in the antibiotic absorption region. Recent work[9-10], in which the properties of 10 different anthracycline antibiotics were compared, shows that the increase in the amplitude of the band upon addition of Cu^{2+} ions is observed only for those antibiotics which contain four reactive oxygen atoms at positions 5, 6 and 11,12.

It is relevant to note that, at the test conditions, only minor changes in the anthracycline absorption spectra are observed upon addition of magnesium, zinc, cadmium or manganese salts Instead, in the case of copper, nickel, iron, palladium or aluminum salts the mentioned changes in the spectra, indicating the formation of complexes of these metal ions with anthracyclines, are marked. However, the amplitude of the CD band is intensified upon the addition of Cu^{2+} ions only. Amplification of the abnormal band in the CD spectrum of liquid-crystalline DNA dispersions by adding anthracyclines and Cu^{2+} ions, reflects the formation of polymeric chelate bridges ("cross-link") (Fig. 2) of the type (DAU- Cu^{2+} -DAU- Cu^{2+} -...- Cu^{2+} -DAU- Cu^{2+} - DAU) between neighboring DNA molecules[11]. These bridges reflect the well-known stereochemical and electronic properties of complexes in which Cu^{2+} ions interact with oxygen atoms of anthracyclines (see, for instance, the presence of the Jahn-Teller effect[12] and fluctional behavior of the configuration of Cu^{2+} complexes[12-13]).

Taking into account the planar geometry of the polymeric chelate (DAU- Cu 2^{+} -DAU- Cu^{2+} -...- Cu^{2+} -DAU- Cu^{2+} -DAU) bridges, it is possible to prove, that the increase in the amplitude of the abnormal band in the CD spectrum upon addition of Cu^{2+} (compare curves 1 and 2 in Fig.1) is related to the rise in concentration of anisotropically oriented DAU molecules in the dispersion. Since polymeric chelate bridges can be formed in any direction starting from any DNA molecule

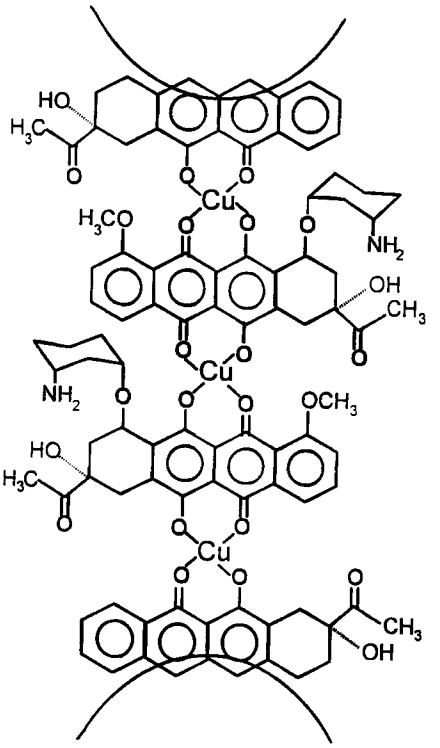

FIG. 2. Model of polymeric chelate bridge between two adjacent DNA molecules in a liquid-crystalline dispersion (a view along the DNA axis). Because the mechanism of DAU fixing onto the surface of DNA remains unknown, the two helical DNA molecules are shown as arcs.

fixed in the liquid-crystalline structure, it is possible to infer that, as a result of interaction of DNA molecules with anthracyclines and subsequent addition of Cu^{2+} ions, a three-dimensional network is formed, in which the neighboring DNA molecules are "cross-linked" by polymeric chelate bridges containing Cu^{2+} ions.

Obviously, the stability of the supramolecular structure depends on the number of polymeric chelate bridges between neighboring DNA molecules. In the presence of a given number of these "cross-links", the factor that can influence the structure and the properties of a molecular construction, is no longer the osmotic pressure of the solvent, but the enthalpy provided by the cross-links. Hence, having a large number of chelate bridges, there is a chance not to need PEG to stabilize the liquid-crystalline dispersion. Under these conditions , one can expect to preserve the optical properties of the supramolecular network,. despite a major change in the solvent osmotic pressure.

To prove that the particles of the liquid-crystalline DNA dispersion "cross-linked" by polymeric chelate bridges exist in the water-salt solution as a three-dimensional molecular construction even after removal of PEG, we have run experiments with the Atomic Force Microscopy (AFM) to measure the size of the particles of the liquid-crystalline DNA dispersion, "cross-linked" by polymeric chelate bridges. Fig. 3 A shows the AFM images of these particles. The x-y particle size distributions, based on direct measurements of the visual shapes of particles in two independent sets of experiments are summarized in Fig. 3B.

The results presented in Fig.3 deserve some comment. First, they demonstrate that the DNA liquid-crystalline particles "cross-linked" by polymeric chelate bridges are stable in water-salt solutions, as predicted. Hence, these conditions one can fix a single DNA liquid-crystalline particle and investigate its properties. Second, despite possible tip-induced flattening of particles, the mean diameter of about 4500 Å corresponds to that obtained by parallel transmission electron microscopy (data not shown). Moreover, the particle diameter of the DNA liquid-crystalline dispersion is similar to that of the particles prepared in PEG-containing solutions without polymeric chelate bridging , as determined by variety of other techniques[6] . Third, the values of sizes measured for x,y,z directions are very similar. Hence, the DNA liquid-crystalline particles, "cross-linked" by polymeric chelate bridges, can be represented as a little elongated spheres with a relative small extent of "spreading" on the supporting film surface. In other words, the three-dimensional structure of the cholesteric liquid-crystalline particle is not altered by "cross-linking".

The formation of polymeric chelate bridges results in fixation of adjacent DNA molecules, i.e. in the formation of a network, in which cholesterically ordered DNA molecules are connected by polymeric chelate bridges. In Fig. 4 a hypothetical scheme of this supramolecular structure is shown.

Finally, Fig.3 shows that it is possible to functionalize a supporting film with a monomolecular layer of DNA liquid-crystalline particles. Such procedure can attract much attention in the field of supramolecular engineering, because it offers a novel way for the fabrication of advanced materials in addition to biosensing devices. Taking into account the high stability of the test molecular networks in water-salt solutions, attempts to immobilize the DNA particles, "cross-linked" by polymeric chelate bridges, were undertaken.

Fig.5 exemplifies the CD spectrum of liquid-crystalline bridged DNA particles, immobilized in agarose gel (curve 2) and then treated with ascorbic acid (curves 3 -5). Comparison of curves 1 and 2 shown in Fig.5 demonstrates that particle immobilization has little effect on their optical properties. In addition, the immobilization of particles does not prevent them from reacting with agents capable to affect the stability of the polymeric chelate bridges (in particular, ascorbic acid). In contrast to Fig.1C, the decrease in the amplitude of the CD band is accompanied by marked displacements of its maximum. According to the theory of the optical properties of colored liquid-crystalline particles[8], this shows that treatment with ascorbic acid results not only in the

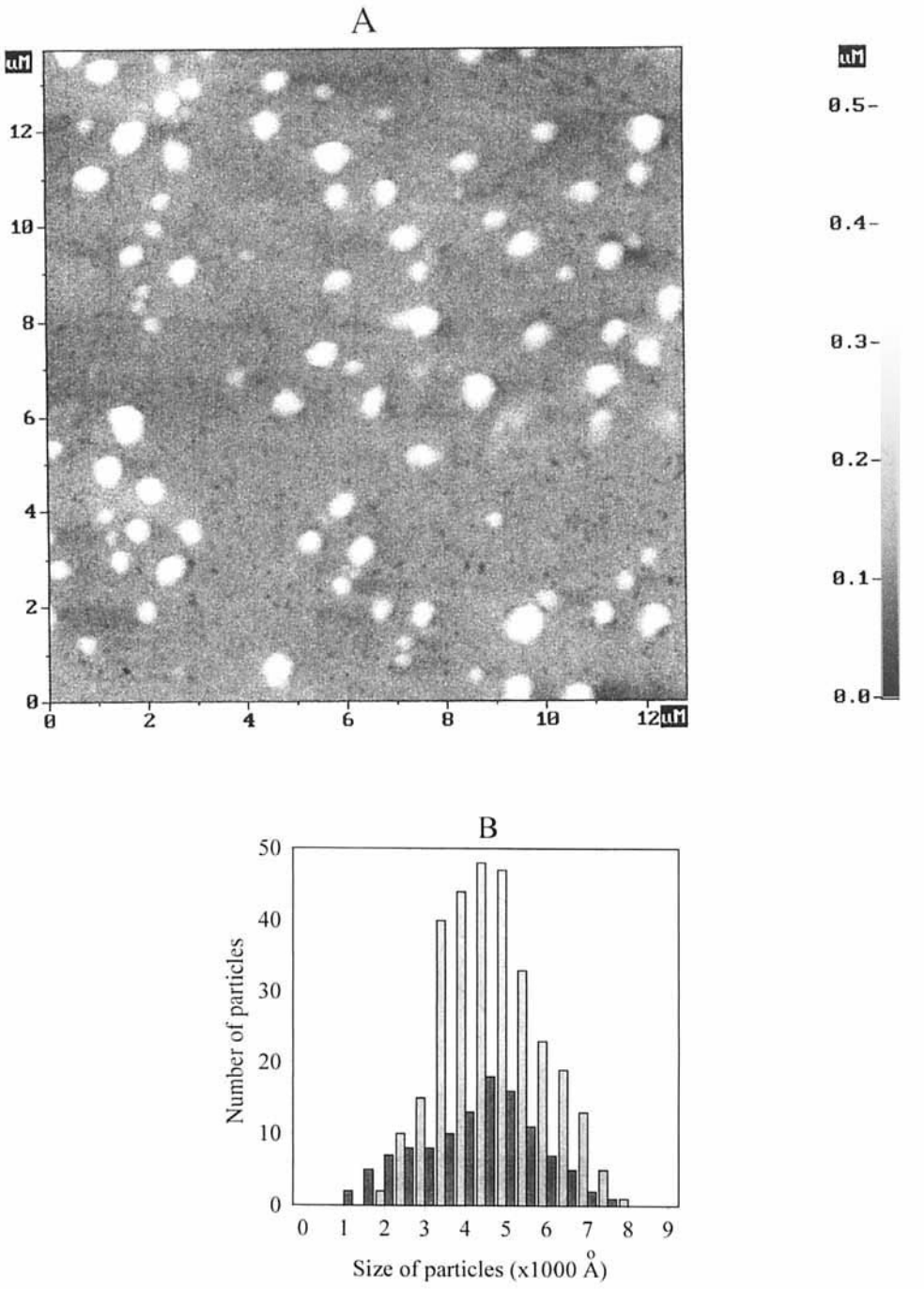

FIG. 3.

A. The Atomic Force Microscope image of the DNA liquid-crystalline particles "cross-linked" by DAU and Cu(II) and immobilized on the surface of the nuclear membrane filter [poly(ethyleneterephtalate)]. The small dark spots correspond to pores in the membrane filter; diameter of pores ≈ 0.10 mm.

B. Size distribution of liquid-crystalline "cross-linked" DNA particles (two independent sets of experiments).

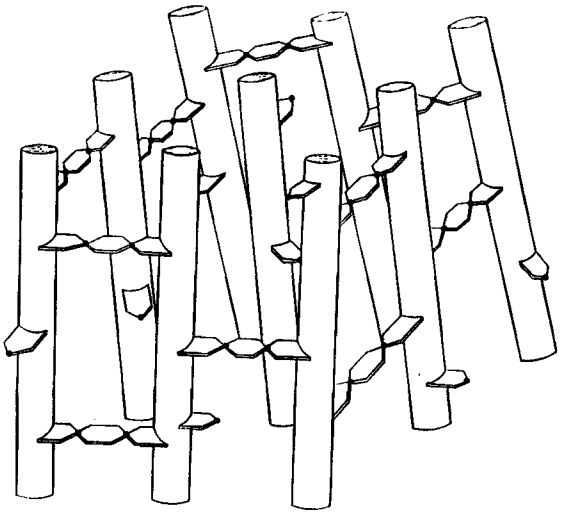

FIG. 4. Scheme of the supramolecular network based on double–stranded liquid-crystalline DNA bridging. Flat chelate bridges (DAU- Cu^{2+} - DAU- Cu^{2} - ...- Cu^{2+} - DAU - Cu^{2+} - DAU) connect DNA molecules shown as rods.

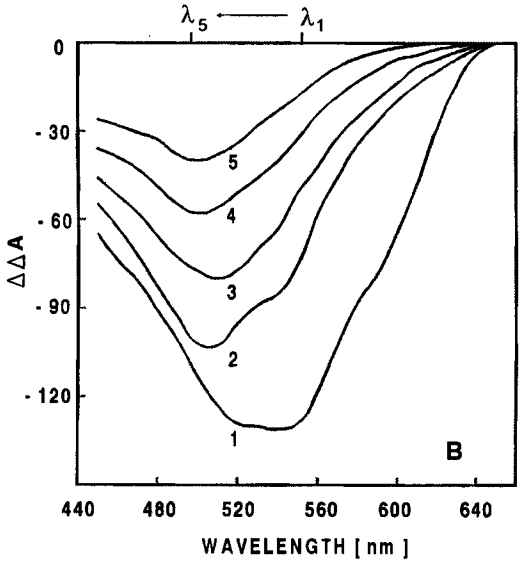

FIG. 5. The CD spectra of the cross-linked DNA liquid-crystalline structure in water-salt solution (curve 1), of the same structure immobilized in 0.6% agarose gel (curve 2) and then treated with ascorbic acid (curves 3 - 5).

$C_{tot\ DAU}$ = 7.08x10 $^{-6}$ M; $C_{tot\ CuCl\ 2}$ = 1.9x10 $^{-6}$ M; C_{DNA} =3.0 mg/ml; C_{PEG} = 17 mg/ml, 0.03 M NaCl + 0.0002 M sodium phosphate buffer, pH 6.67; $C_{tot\ Ascorbic\ acid}$ = 5 x10 $^{-6}$ M; time of ascorbic acid treatment: curve 2 - 0 min; curve 3 - 4.3 min; curve 4 - 10.7 min; curve 5 - 25.5 min;

Optical path 0.5 cm; the dichroic absorption change ΔΔA is given in mm; 1 mm = 10^{-6} optical units.

cleaving of polymeric chelate bridges, but also in the total collapse of the three-dimensional arrangement of liquid-crystalline particles.

The data obtained here are in agreement with the role of polymeric chelate bridges as the main stabilization factor in the absence of PEG. Indeed, when the bridge is broken, a transition occurs from the liquid-crystalline to the isotropic state [6].

In addition to allowing a detailed investigation of the physico-chemical and the electrochemical properties of the small size, stable particles formed by bridging together double-stranded DNA molecules, our results open a gate for an application of the "cross-linked" DNA particles as biosensing units for the detection of numerous compounds, including drugs, able to interfere with the formation of bridged structure. Since even a few analyte molecules strongly affect the liquid-crystalline state of the ordered network, very high sensitivity is to be expected for the proposed application.

Finally, functionalization of the supporting film with a monolayer of the DNA liquid-crystalline structure should attract attention in the field of supramolecular engineering. Indeed, such technique offers a novel procedure for fabrication of advanced materials as well as of new types of sensing units.

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