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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Liquid Crystal Dispersions of DNA Complexes with Gadolinium As a Possible Basis for Neutron-Trapping Therapy

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Neutron-trapping therapy (NTT) is a method for treating malignant tumors based on neutron trapping by nonradioactive elements exposed to thermal neutrons [1]. Gadolinium (Gd) is one of these nonradioactive elements. In the case of Gd NTT, a ^{157}Gd preparation administered *in vivo* is used for the neutron-trapping reaction. The neutron-trapping nuclear reaction (^{157}Gd $(n, \gamma)^{158}$Gd) is accompanied by the appearance of γ quanta and Auger electrons destroying the tumor tissue [1] and damaging the DNA of neoplastic cells [2]. The key factor of the success of Gd NTT is to obtain nontoxic [3, 4] molecular constructions [5–8] capable of maintaining a high concentration of Gd^{3+} after the substance has been administered into the tumor. Special attention is paid to various nanoparticles containing Gd^{3+} [4, 9, 10].

Microscopic particles of cholesteric liquid crystal dispersions (cLCDs) of double-stranded DNA (dsDNA) containing ions of rare earth elements have been obtained for the first time. These particles considerably differ from classic DNA cholesterics in a number of properties. The local concentration of rare earth elements in a particle may be as high as 200 mg/ml. The particles of the dispersion of the DNA–gadolinium complex retain their properties for a long time. The combination of microscopic sizes, the high concentration of gadolinium in the particles, and stability makes these particles promising for practical applications.

We used dsDNA cLCDs as a system ensuring high local concentrations of gadolinium.

Figure 1 shows an example of a circular dichroism (CD) spectrum of linear dsDNA treated with $GdCl_3$. As Gd^{3+} ions interacted with DNA, the amplitude of the positive band at λ = 280 nm in the CD spectrum decreased, and the band changed the sign at a $GdCl_3$ concentration of ~2.5×10^{-5} M. Addition of La^{3+}, Nd^{3+}, or Sm^{3+} ions caused a similar change in the shape of the CD spectrum. The same distortion of CD spectra was earlier observed in experiments on the interaction of bi- and trivalent metal ions with DNA [11, 12]. It is explained in terms of the formation of chelate complexes of ions and base pairs accompanied by alter-

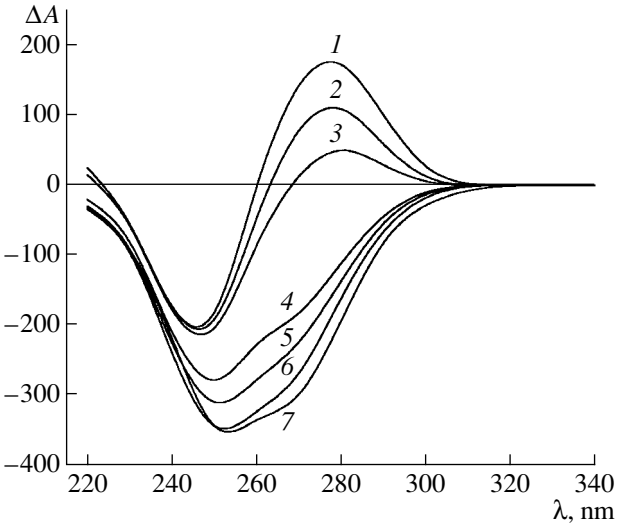

Fig. 1. The CD spectra of linear dsDNA in the absence and in the presence of $GdCl_3$: (*1*) original dsDNA in the absence of $GdCl_3$; (*2–7*) addition of 1×10^{-5}, 2×10^{-5}, 3×10^{-5}, 4×10^{-5}, 5×10^{-5}, and 6×10^{-5} M $GdCl_3$, respectively. C_{DNA} = 30 μg/ml, $C_{NaCl} = 5 \times 10^{-4}$ M, $\Delta A = (A_L - A_R) \times 10^6$ opt. un.

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ations in the dsDNA secondary structure (even to the extent of strand separation) [13, 14]. It is obvious that, despite the interaction of rare earth metal ions with linear dsDNA molecules, it is impossible to obtain their high concentration in this system.

This is not so with a dsDNA cLCD. Upon phase exclusion of dsDNA molecules from water–polymeric solutions, these molecules form LCDs whose properties are easily controlled by varying the osmotic pressure of the solution or the characteristics of DNA molecules [15]. Not only are neighboring dsDNA molecules in a cLCD particle orderly arranged, but, in addition, their local concentration may be as high as 400 mg/ml [15]. As an example, Fig. 2 shows the comparison between the CD spectrum of the original dsDNA cLCD and the CD spectra of this dispersion after $GdCl_3$ treatment. The amplitude of the negative band at $\lambda \sim 270$ nm, which is typical of dsDNA cLCDs, was increased, and its maximum was shifted to the red region. The change in the shape of the CD spectrum indicates that Gd^{3+} ions (as well as La^{3+}, Nd^{3+}, and Sm^{3+} ions) interacted with dsDNA forming cLCD. Several considerations should be taken into account when analyzing the observed effect. First, if the interaction of these ions with dsDNA in cLCD particles had substantially disturb the mutual orientation of bases, this, in principle, would have only decreased the amplitude of the negative band in the CD spectrum. Second, the separation of individual strand of dsDNA in cLCD particles, which could have accompanied the interaction of rare earth ions with dsDNA is impossible for steric reasons. Third, the band amplitude was increased at a Gd^{3+} concentration substantially higher than that necessary for distorting the spectrum of linear dsDNA (Fig. 1). Finally, the estimation of Gd^{3+} concentration in dsDNA cLCD particles based on measuring their magnetic susceptibility showed that there was one Gd^{3+} ion per dsDNA phosphate group, i.e., the negatively charged phosphate groups of dsDNA molecules were neutralized by the positively charged Gd^{3+} ions. These data suggest that the amplification of the negative band in the CD spectrum was determined by a factor other than the aforementioned factors distorting the CD spectrum of linear dsDNA. Theoretically, changes in the parameters of the spatial structure of dsDNA cLCD particles, such as the change in the pitch (P) of their cholesteric structure, are the most probable reason for the observed effect (Fig. 2). The formation of an almost insoluble complex between Gd^{3+} ions and the phosphates of dsDNA [12, 13] influences the distribution of charges over the surface of dsDNA molecules, which may cause changes in P. Calculations showed that a decrease in P corresponded to the observed increase in the amplitude of the negative band in the CD spectrum. In addition, the summary surface charge of dsDNA cLCD particles stabilized them, thereby preventing the particles from coalescence into a single phase. Indeed, despite the thermal "training" of the precipitated dsDNA cLCD particles treated with $GdCl_3$, the homogeneous phase

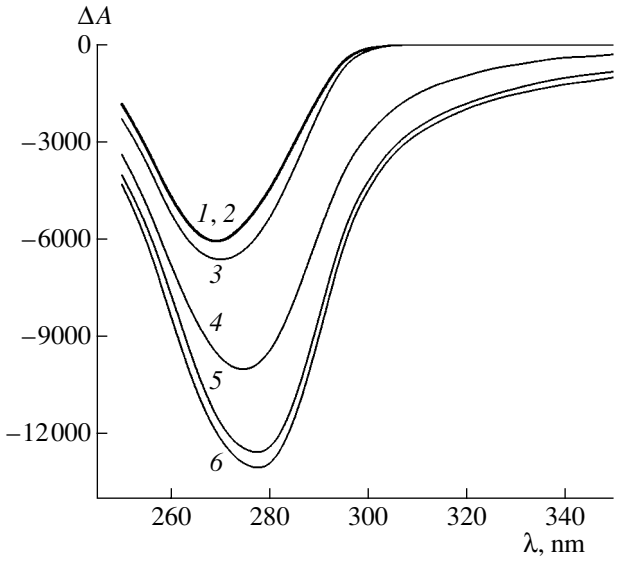

Fig. 2. The CD spectra of dsDNA cLCD in the absence and in the presence of $GdCl_3$: (*1*) original dsDNA cLCD in the absence of $GdCl_3$; (*2–6*) addition of 0.37×10^{-3}, 0.74×10^{-3}, 1.48×10^{-3}, 2.91×10^{-3}, and 4.31×10^{-3} M $GdCl_3$, respectively. C_{DNA} = 15 μg/ml, C_{PEG} = 170 mg/ml, $C_{NaCl} = 3 \times 10^{-1}$ M, $\Delta A = (A_L - A_R) \times 10^6$opt. un.

with a characteristic texture that could have been observed using a polarization microscope was not formed. In this respect, the resultant particles differed from the original dsDNA cLCD particles, which readily form a phase with a characteristic "fingerprint" texture after thermal training [15]. Although the precipitated dsDNA cLCD particles treated with $GdCl_3$ intensely scattered X-rays, the isotropic behavior of the particles precluded the formation of a maximum corresponding to the mean distance between dsDNA molecules in cLCD particles on the scattering curve (in contrast to the precipitate of the original dsDNA cLCD characterized by a d_{Bragg} value of 31–32 Å [15]). Therefore, the characteristics of dsDNA cLCD particles after $GdCl_3$ treatment considerably differed from the original ones.

The presence of independent, almost insoluble dsDNA cLCD particles after $GdCl_3$ treatment was confirmed by their visualization. Figure 3 shows these particle immobilized on a nuclear membrane filter. The mean size of the particles was 4500–5000 Å. (The original dsDNA cLCD particles did not exist in the absence of osmotic pressure of the solvent; therefore, it was merely impossible to immobilize and, hence, visualize them under the conditions used.) The visualization of single particles indicated that the "liquid" packing pattern of dsDNA molecules in dsDNA cLCD particles was replaced by a rigid spatial structure after the $GdCl_3$ treatment of the particles. Note that, according to the results of our calculations, the local concentration of

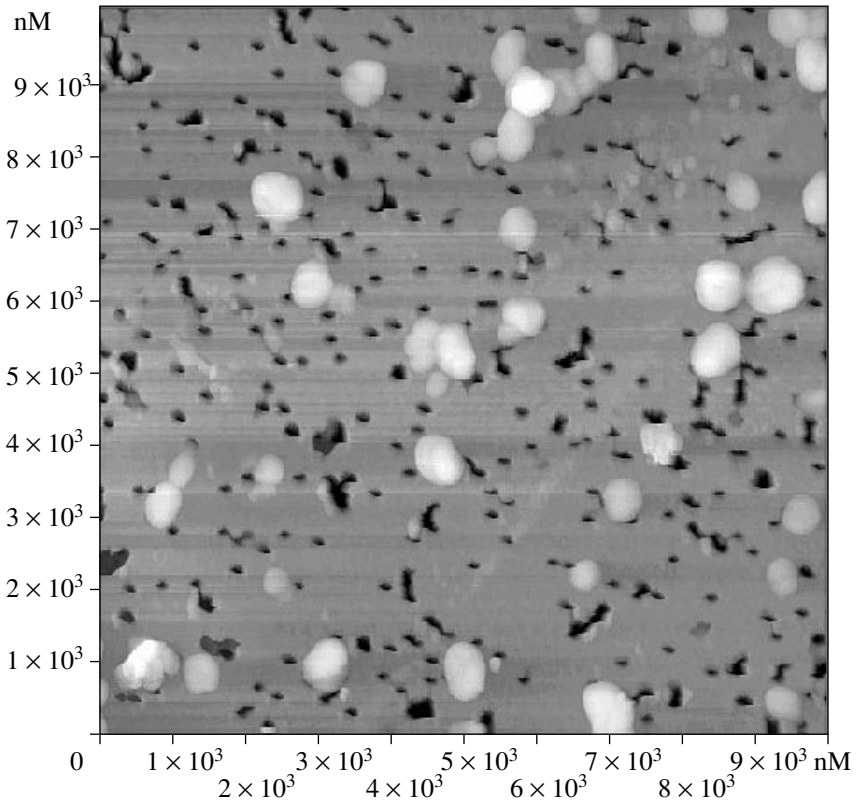

Fig. 3. Visualization of dsDNA cLCD particles treated with $GdCl_3$ and immobilized on a nuclear membrane filter. $C_{CdCl_3} = 2.33 \times 10^{-4}$ M, $C_{DNA} = 1.1$ μg/ml, $C_{PEG} = 17$ mg/ml, $C_{NaCl} = 3 \times 10^{-2}$ M. Mean diameter of pores in the filter, 0.25 μm. The image was obtained using an atomic power microscope.

Gd^{3+} in dsDNA cLCD particles was as high as 200 mg/ml.

Thus, the high abnormal optic activity of dsDNA cLCD particles treated with Gd^{3+} and the high, easily controlled concentration of Gd^{3+} contained in these particles make it possible to use dsDNA cLCD particles not only in medicine (as a basis for neutron-trapping therapy), but also in technology (as an object with stably abnormal optic characteristics).

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