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Nanoconstructions based on double-stranded nucleic acids

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Abstract

We describe the formation and properties of nanoconstruction that consists of the double-stranded DNA molecules located at distance of 35–50 Å in the spatial structure of particles of their cholesteric liquid-crystalline dispersions and cross-linked by artificial nanobridges. The resulting nanostructures possess the peculiar spatial and optical properties.

Keywords: Nanostructure; Nucleic acids; Molecular design; Liquid-crystalline dispersions; Chelate complexes; Nanobridges; Circular dichroism

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1. Introduction: the creation of nanoconstructions based on ds nucleic acids

Nanodesign based on the double-stranded (ds) nucleic acids (NAs), i.e. directed creation of the three-dimensional, spatial constructions with the tailored properties,"building blocks" of which are dsDNA molecules or their complexes with biologically active compounds, is a topic of current theoretical and experimental interest [1–3]. The dsDNA (RNA) nanoconstructions (NaCs) are of significal practical importance, as a minimum, from two points of view. First, NaCs with adjustable spatial parameters can be used in bioelectronics and biosensorics [4,5]; second, the DNA NaCs can be used for the delivery of genotoxicants or biologically relevant compounds into eucaryotic cells.

The very possibility of using dsNA for formation of NaCs with controlled parameters is based on a few properties characteristic of NA molecules only:

(a) the short helical molecules of dsNA with lengths of the order of 100–1000 Å have a high local rigidity at standard solvent properties, that allows such molecules to be used as "building blocks" without change in their physical properties;
(b) flexible single-stranded NA not only recognizes a complementary strand but also hybridizes with it to form a strong complex; this causes a change in the spatial structure of the single-stranded NA and the formation of a rigid double-stranded molecule;
(c) creation of sticky ends in dsNA combained with an appearance of "branch-point", because of the presence of specific sequences of nitrogen bases in this structure, makes it possible to branch the resulting NaCs built thereof;
(d) in the case of rigid dsNA molecules, their properties and the character of intermolecular interaction under different conditions can be programmed, making it possible to tune the peculiarities of designed spatial constructions;
(e) nitrogen bases in the spatial NA constructions retain their capability not only to interact with different chemical substances or biologically active compounds but also to orient them with respect to the long axis of NA molecule, which imparts additional chemical reactivity to the whole construction.

Abbreviations: CD, circular dichroism; dsNA, double-stranded nucleic acid; DAU, anthitumor anthracycline antibiotic-daunomycin; LCD, liquid-crystalline dispersion; NaC, nanoconstruction; NA, nucleic acid

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To date, two main strategies have been described for designing NA nanostructures.

First strategy, which takes into account points "b–d" above and could be named conventionally as a successive design or step-by-step design, based on successive modification of initial NA molecule. According to approach suggested and developed by Seeman [6], newly-synthesized, rigid, ds fragments of DNA (polynucleotides) containing not only sticky ends but also particular base sequences were used as building blocks with adjustable properties. By the technique of oligonucleotide synthesis in combination with a set of restriction endonucleases and ligases used for cleavage and linking of desired sequences, the authors created nanostructures having the shapes of cube, octahedron, hitched octahedron, dodecahedron, etc., whose stiffening ribs are DNA molecules [7].

There are a few additional approaches, which may be called "supplementary" to the approach suggested by Seeman. One of such technique is based on the use of molecule composed of two single-stranded self-complementary oligonucleotides, the ends of which are bound to each other via a rigid chain of two *p*-(2-hydroxyethyl)-phenylethynylphenyl spacers linked to a tetrahedral carbon atom [8]. The hybridization of oligonucleotide fragments, which results in formation of ds structure, is accompanied by the emergence of a set of multi-arm star-shaped NaCs. The rigidity of the constructions is provided by alternation of hydrocarbon fragments in definite spatial conformation and ds oligonucleotides. Another technique by Niemeyer et al. [9] is based on the use of bis(biotinylated) DNA molecule and biotin-binding protein—streptavidin. This allows nanostructures of closed loop shape to be made using reactions of complex formation.

An approach to nanodesign used almost simultaneously by Mirkin et al. [10] and by Alivisatos et al. [11] uses as the building blocks the single-stranded NA linked to the particles of colloidal gold. The addition of "foreign" NA, whose base sequence is complementary to the initial NA, leads not only to the formation of rigid dsNA by hybridization, but also to the formation of a three-dimensional construction containing spatially ordered gold particles and dsNA fragments.

It should be noted that the issue of the practical application of NaCs created from a single dsNA molecule via step-by-step technology seems to be determined by the tasks to be solved by researchers. The retention of physicochemical properties of NA during the nanodesign opens the way to insert atoms or molecules of different compounds ("guest" molecules) into initial NA molecules or resultant NaCs. In particular, NaCs whose properties depend on the length of NA molecules and the size of inserted metal particles could be used as biosensing elements [5,12]. Moreover, the NaCs could be used in nanoelectronics. If three-dimensional ordering of single NaCs, i.e. their crystallization, will be achieved, it is likely that compounds poorly crystallizable under common conditions, which are introduced somehow into the NaC, could be crystallized within NaC. However, such an ordering itself is a complicated task, which is not solved to date.

Second strategy of creating NaCs containing dsNA molecules suggested by us earlier [13] takes into account points "a, d, e" above. This strategy *differs in principle* from all the above variants of the step-by-step strategy, because our strategy makes use of the liquid-crystalline dispersions (LCD), rather than single NA molecules, resulting from the phase exclusion of dsNA molecules from aqueous polymeric solutions. As a result of phase exclusion, rigid ds molecules of NA (or polynucleotides) form particles composed of about 10^4 molecules; each particle is about 5000 Å in size, which was evaluated by several experimental techniques (the low speed sedimentation, the UV-light scattering, the laser correlation spectroscopy, etc.) and confirmed by theoretical calculations [14]. According to the X-ray study, NA molecules are ordered in the particle at distances of 30–50 Å, i.e. they acquire the properties of a crystal, but molecules in the neighboring layers are mobile, i.e. they retain the properties of a liquid. Such combination of properties allows this structure to be called as "liquid-crystalline" (see reviews [14,15] and early references cited therein). The most important features of dsNA LCDs are well established now. First, LCDs exist under certain boundary conditions, which are determined, in particular, by solution ionic strength, by the value of osmotic pressure of aqueous polymeric solution, etc. The osmotic pressure, which depends on polymer concentration in solution, determines the distance between the NA molecules in a particle. Second, spontaneous constraint of diffusional degrees of freedom of neighboring NA molecules takes place upon phase exclusion. Third, the combination of geometrical and optical anisotropy of NA molecules causes each next layer formed by NA molecules in the structure of the liquid-crystalline particle (a so-called 'quasinematic layer') to be turned through a certain angle with respect to previous one, i.e. spatially twisted or a so-called "cholesteric structure" of the particle arises. Violation of the boundary conditions results in the disappearance of the spatial structure of particle. Fourth, because NA molecules contain chromophores (nitrogen bases absorbing in the UV-region of the spectrum), the resulting cholesteric may be named as "colored" cholesteric. Since the bases are virtually perpendicular to the long axis of NA molecules forming adjacent layers in the structure of the cholesteric, theory [16] predicts an appearance of an intense (abnormal) band in the circular dichroism (CD) spectrum in the bases absorption region, which is indeed observed experimentally. It should be noted that theory [16] imposes no limitations on the number of chromophores that could be introduced into the NA structure in the same manner, i.e. one could expect an appearance of the abnormal CD bands preferably for compounds intercalating between the NA base pairs. This means that there is an analytical "instrument" capable of monitoring the finest variations in the properties of NA molecules and cholesterics produced thereof. And finally, the chemical reactivity of NA molecules remains unchanged upon formation of the LCD particles; this opens