Nanotechnology Based on Spatially Fixed DNA (RNA) Molecules

M. A. Zakharov, Yu. M. Yevdokimov, S. G. Skuridin, Yu. D. Nechipurenko.

Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, 119991 Moscow, Vavilova str. 32, Russia;

ABSTRACT

We describe the formation and the properties of a nanoconstruction that consists of the double-stranded molecules of nucleic acids (or synthetic polynucleotides) located at a distance of 35 Å in the spatial structure of particles of their cholesteric liquid-crystalline dispersion and crosslinked by artificial nanobridges. The resulting nanostructures possess the peculiar physicochemical properties.

Key words: nanostructure, nucleic acids, molecular design, liquid-crystalline dispersions, chelate complexes, nanobridges, circular dichroism

INTRODUCTION

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 ds DNA molecule or their complexes with biologically active compounds, is a topic of current theoretical and experimental interest [1]. The ds DNA (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 [2]; second, the DNA NaCs can be used for the delivery of genotoxicants or relevant biologically active compounds into animal cells.

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

(a) the short helical molecules of ds NA 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 chain 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 ds NA 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 ds NA 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 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;

To date, several strategies have been described for designing NA nanostructures that take into account points „a-d“ above and most of them could be named conventionally as a successive design or step-by-step design, based on successive modification of initial NA molecule. [3-6]

Our strategy of creating NaCs containing ds NA molecules 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 ds NA molecules from aqueous polymer 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. NA molecules are well-ordered in the particle at distances of 30–50 Å, i.e., they acquire the properties of a crystal, neighboring molecules forming layers where these molecules are mobile, i.e., they retain the properties of a liquid. Such a combination of properties allows this structure to be called as a “liquid-crystalline” (see review [7] and early references cited therein).The most important features of LCDs of ds NAs 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 polymer solution, etc. The osmotic pressure, which depends on polymer concentration in solution, determines the distance between DNA molecules in dispersion particle.

Second, spontaneous phasing of neighboring NA molecules and constraint of diffusional degrees of freedom of these molecules takes place upon phase exclusion.

Third, the combination of geometrical and optical anisotropy of NA molecules causes each next layer of NA

molecules to be turned through a certain angle with respect to previous one, i.e., spatially twisted or a so-called "cholesteric structure" of liquid crystal arises. Violation of the boundary conditions results in the disappearance of the spatial structure of dispersion particle.

Fourth, because NA molecules contain chromophores (nitrogen bases absorbing in the UV-region of the absorption 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 predicts an appearance of a very intense (abnormal) band in the circular dichroism (CD) spectrum in the bases absorption region, which is indeed observed in experiment. It should be noted that theory 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 the way to purposeful alteration of the properties of these molecules.

Consideration of the above points reveals the fundamental possibility for spatial fixation of the neighboring, closely-located and fairly low-mobile NA molecules by formation of nanobridges (crosslinks) between these molecules, i.e. it is possible to create NaC, whose properties can be specified in advance by controlling both of the properties of NA molecules and of the solvent used. This means that our technology provides a possibility to create NaCs with preset properties. Besides, the use of the LCD particles for nanodesign automatically solves the problem of ordering of both neighboring NA and guest molecules, which is not solved yet in the case of step-by-step strategy.

Thus, there is a possibility to use NA molecules within the LCD particles as building blocks with adjustable properties. At the first stage of our study, we inserted nanobridges (crosslinks) with adjustable properties between NA molecules in LCD particles. The crosslinking of NA molecules by chelate complexes based on anthracycline antibiotics has been used in this work to create NaCs.

THE CREATION OF NANOCONSTRUCTIONS BASED ON THE LIQUID CRYSTALLINE DISPERTIONS OF DS Nas AND THEIR CD SPECTRA

NaCs based on ds NAs or synthetic polyribonucleotides were obtained by three-stage scheme, described in detail earlier [8].

In Fig.1 the CD spectra of an initial DNA cholesteric LCD (curve 1), this dispersion treated with DAU (curve 2) and then by $CuCl_2$ solutions (curves 3), are compared.

Fig.1 show, that the formation of the DNA LCD particles result in an appearance of the intense band in UV-region of the spectrum (curve 1), i.e. in the region of absorption of the DNA nitrogen bases (chromophores having an absorption band in the UV-region of the spectrum).

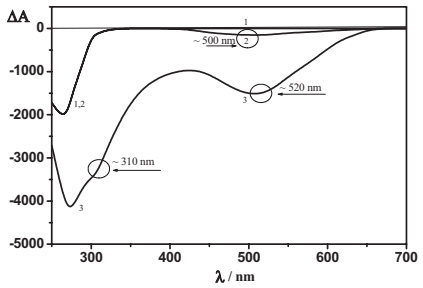

Fig. 1

This band testifies to a specific polarization of electron transitions of nitrogen bases in respect to an axis of DNA molecules forming "quasinematic" layer of a cholesteric. The negative sign of the band in the CD spectrum ($\lambda \sim 270$ nm) of LCD of DNA and polynucleotides of the B-family indicates the left-handed twist of the spatial structure of particles of cholesteric LCD resulting from phase exclusion of the right-handed molecules.

Addition of DAU to the DNA LCD is accompanied by the appearance of the band located in the absorption region of DAU. Amplitude of the band at $\lambda \sim 500$ nm (curve 2, Fig.1) exceeds essentially the amplitude, reference for a "molecular" circular dichroism of isolated DAU molecules. The negative sign of the band at $\lambda \sim 500$ nm, which similar to the sign of the band, reference for the initial cholesteric DNA LCD, shows that the orientation of DAU molecules in respect to long axis of the DNA helix is coinciding with orientation of the DNA base pairs. (One can add, that the reactive groups of DAU (keto-oxygen, *peri*-OH groups) become unavailable for chemical reactions upon intercalation).

However, upon formation of "external" complex, the reactive groups of all anthracyclines, which are not built-in between the DNA base pairs, are accessible to the chemical reactions. Indeed, these compounds, owing to their chemical structure, can form chelate complexes with the metal ions. Chelate complexes produced by Cu^{2+} ions are of particular interest. The bands at $\lambda \sim 500$ nm and $\lambda \sim 300$ nm are characteristic of the CD spectrum of linear, isotropic (DAU-Cu^{2+}) complexes; this reflects the existence of low- ($\lambda \sim 500$ nm) and high-($\lambda \sim 300$ nm)-energy electronic transitions in DAU moieties of the complexes. These two bands are maintained at the formation of a complex between the linear DNA and DAU. An addition $CuCl_2$ solution to the DNA LCD treated by DAU and having an equilibrium value of the amplitude of the band at $\lambda \sim 500$ nm results not only in a manyfold increase (amplification) of this band, but also a

band located in the UV-region of the spectra (curve 3, Fig.1).

Fact of the amplification of the band in UV-region shows that the DAU molecules and copper ions are forming a complex, which optical properties differ from properties of intercalation complex between DNA and DAU molecules (compare curve 2 and curve 3 in Fig.1).

The manyfold amplification of the bands at $\lambda \sim 505$ nm $\lambda \sim 310$ nm in the case of LCD of DNA indicates the appearance of an additional (along with intercalation) type of anisotropic arrangement of DAU molecules in proximity to DNA molecules. The anisotropic arrangement of (DAU - Cu^{2+}) complexes , which differs from common intercalation of DAU and causes the amplification of the 505 and 310 nm bands in the CD spectrum of the NA LCD, may appear in two different situations.

First, one may suppose that, owing to stacking interaction between DAU molecules this is caused by the formation of vertical stacks (n-mers) from DAU molecules near to the NA surface in the structure of the LCD particles. This means that a shell of DAU appears in proximity to the surface of NA molecule where a portion of DAU molecules is bridged by Cu^{2+} ions. It is obvious that the direction of the vertical axis of the resulting structure of DAU n-mers coincides with the direction of the NA long axis. Although the question of the steric hindrances arising upon such an arrangement of neighboring DAU molecules, in particular, the location of sugar residues, remains open, the orientation of DAU molecules in this structure may be very similar to the orientation of NA base pairs. Second, one may suppose that complexes (DAU - Cu^{2+}) are appeared between the neighboring NA molecules. It should be noted that, in principle, the beginning of the nanobridge may be not only a DAU molecule forming the "external" complex but also a Cu^{2+} ion bound to NA bases. The direction of the long axis of the nanobridges, formed by [DAU- Cu^{2+}] complexes, proves to be perpendicular to the direction of the long axis of NA molecules, although the orientation of DAU molecules is close to that of NA base pairs. Additional experiments testify the second suggestion.

To prove that the particles of the ds DNA LCD crosslinked by nanobridges can exist in the water-salt solution as even after removal of PEG, we have run experiments with the Atomic Force Microscopy (AFM) to measure the size of the particles.

Fig.2 demonstrates, as an example, 2D image of the ds DNA LCD particles treated sequentially with DAU and $CuCl_2$ solutions, and immobilized on nuclear membrane filter. This is possible only if neighboring NA molecules are crosslinked via nanobridges, which stabilize the cholesteric structure of dispersion particles. Thus, the reason for the increase in the amplitude of bands located in different regions of CD spectrum of LCD of NA consecutively treated by DAU and $CuCl_2$ is the formation of nanobridges between neighboring NA molecules, i.e. the formation of the NA nanoconstruction (NaC).

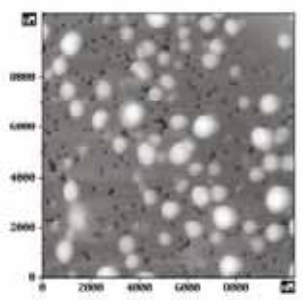

Fig. 2

THE PREREQUISITES FOR FORMATION OF NANOBRIDGES BETWEEN DOUBLE-STRANDED NUCLEIC ACIDS

1. The structure of anthracycline antibiotics.

The analysis of more than 10 DAU analogs, that differ by the presence and position of substituents at the aglycon, showed that the presence of four reactive atoms of oxygen in the 5,6 and 11,12 positions of anthracyclines, is one of essential prerequisite for the amplification of optical activity upon building of nanobridges.

2. The distance between ds NA molecules.

The result of magnetometric measurements showed that six Cu^{2+} ions are existing in the content of nanobridge. Taking into account the DAU structure, this allows us to suppose that each nanobridge between the neighboring NA molecules has spatial structure shown in Fig. 3. The stereochemistry of ds NA molecules and symmetry of the nanobridge permits one to maintain, that for the formation of nanobridges a few parameters related to NA molecules are essential. First, it is the distance between NA molecules, which is directly determined by the osmotic pressure of a solvent. The ordered ds NA molecules in a quasinematic layer should possess an abiity for freely rotation around the long axis, i.e., the diffusion behaviour of NA molecules in a quasinematic layer can be similar to that of in a liquid. A very close packing NA molecules will restrict the formation of nanobridges with the shown symmetry (Fig.3). Second, the helical parameters of NA molecules, but not the stereochemical details of nitrogen bases are important. This, in turn, means, that the nanobridges will be formed between any ds NAs, despite of the differences in their nucleotide composition. If one can take the ds NA molecules, fixed in the LCD structure, as the „building" blocks, then the formation of nanobridges will happen with the practically identical efficacy for the case of ds DNA or ds RNA.

Hence, the specific distance between ds NA molecules is a prerequise for the nanobridge formation with a symmetry shown in Fig.3.

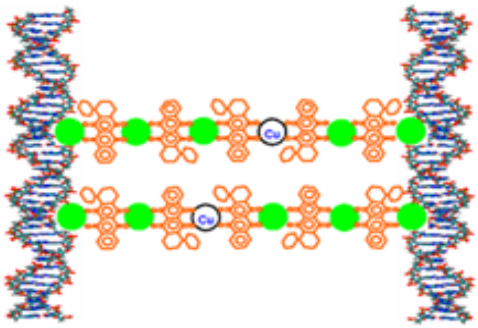

Fig. 3

3. The "phasing" of NA molecules

One can supose that the rigid, flate nanobridges (DAU- Cu^{2+}) can be formed between the closest neighbour NA molecules (Fig. 4) located in one quasinematic layer, if these molecules are sterically "phased" in this layer.

Because of the spiral structure of ds NA, in order to join the same chemical groups in the content of the neighboring NA molecules by the nanobridge with a fixed symmerty, it is necessary to turn the NA molecule "2" around its long axis on 180^0 in respect to molecule "1" (Fig.3,4). This means, that nanobridging will be met only at definite positions of ds NA molecules within quasinematic layers of particles of LCD, when spatial adjustment of position of neigboring NA molecules (the "phasing") is spontaneously realized. For the "phasing" NA molecules, such a degree of diffusion fredom for both NA molecules can exist, which is enough for a rotation of molecules around their long axes.

Hence, the "phasing" of NA molecules, is the final prerequisite for the formation of nanobridges.

The prerequisites above show that the formation of nanobridges between neighboring ds NA molecules is a very delicate stereochemical process, which could be realized under rather strict conditions.

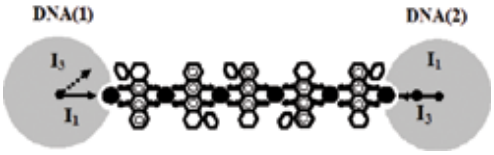

Fig. 4

ON THE PRACTICAL APPLICATION OF NANOCONSTRUCTIONS

The NaCs created by us are of interest for various areas of practical application. Attention may be paid to the fact that the hypothetical structure of the NaC (Fig. 3) contains a "sensing element," which is represented by any component of nanobridge. Because of the particular sterical conformation of the chelate complex formed by DAU molecules and Cu^{2+} ions, this complex can be destroyed under the action of different factors. This may cause disappearance of structural integrity of the bridge between NA molecules within the NaC, which results finally in the disintegration of NaC as a whole.This process must be accompanied by a decrease in the abnormal optical activity. Under definite conditions, the degree of decrease in the amplitude of the band in the CD spectrum of the NaC is proportional to the concentration of chemical or biologically active compound destroying the nanobridge. This means that the NaC can be used as a biosensing unit sensitive to the presence of some chemical or biologically active compounds in a liquid to be analyzed and the change in the optical properties of this biosensing unit can be easily detected.

In conclusion, one can say, that the NaCs, built by us, represent a new type nanobiomaterial. The properties of this material depend on properties of nanobridges and they can be adjusted according to the requirements of the consumer

REFERENCE

1. Service R.F., "DNA ventures into the world of designer materials", Science, 277, 1036–1037, 1997.
2. Robinson B.H., Seeman N.C., "The design of a biochip: a self-assembling molecular-scale memory device", Protein Eng., 1987.
3. Seeman N.C., "Nucleic acids junctions and lattices", J. theor. biol., 99, 237-247, 1982.
4. Chen J., Seeman N.C., "The synthesis from DNA of a molecule with the connectivity of cubes"., Nature, 350, 631-633, 1991.
5. Shi J., Bergstrom D.E. "Assembly of novel DNA cycles with rigid tetrahedral linkers"., Angew. Chem. Int. Ed., 36, 111–113, 1997.
6. Niemeyer C.M., Adler M., Gao S., Chi L., "Supramolecular nanocircles consisting of streptavidin and DNA", Angew. Chem. Int. Ed., 39, 3056–3059, 2000.
7. Yevdokimov Yu.M., Skuridin S.G., Lortkipanidze G.B., "Liquid-crystalline dispersions of nucleic acids", Liq. Crystals, 12, 1-16, 1992.
8.Yevdokimov, Yu.M., Salyanov,V.I., Zakharov M.A., "A novel type of microscopic size chip based on double-stranded nucleic acids", Lab on a Chip, 1, 35–41, 2001.