C-Ag\(^{+}\)-C based repetitive DNA sequence

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Abstract. DNA is a convenient and well-studied tool for nanostructures fabrication. Metal-mediated hybridization of DNA strands opens up new possibilities for nanobiotechnology. In this work, we studied the possibility of long DNA formation from short ones by gluing them through the formation of C-Ag\(^{+}\)-C complexes. Such long formations were investigated using static light scattering and atomic force microscopy. It was found that the duplexes can efficiently be linked in the presence of silver ions if the length of the cytosine sequence exceeds 6 nucleobases.

1. Introduction

Besides being a carrier of genetic information, DNA also found a lot of applications in the field of nanotechnology due to the high specificity and strength of the interaction between the complementary nucleosides [1].

To expand the possibilities of DNA nanotechnology, in addition to natural base pairs, various modifications of base pairs were also created. Metal-mediated base pairing is a promising approach that improves DNA stability [2] and conductivity [3,4]. In metal-mediated base pair, the hydrogen bonds between the nucleobases are replaced by a coordination bond through metal ions. One of the first works, describing this kind of bonding was performed by Katz et al. and Y. Miyake et al. and was devoted to a formation of T-Hg-T metal-mediated pairs [5,6]. Later Ono et al. and Torigoe et al. [7,8] reported the highly specific formation of C-Ag\(^{+}\)-C base pairs for a case of only one C-C mismatch pair located in the middle of the complementary duplex and an increase in thermodynamic stability of such duplex.

Of particular interest are not only single mismatches but the whole sequences of metal-mediated base pairs. Such systems have been described in detail in the work of Swasey et al., in which homo-base DNA oligomers were shown to be jointed through the formation of C-Ag\(^{+}\)-C pairs [9].

Along with the fact that the internal C-Ag\(^{+}\)-C mismatch stabilizes the DNA duplex, the terminal C-Ag\(^{+}\)-C mismatch does not stabilize the DNA duplex in the same way [10]. More detailed information on the terminal C-Ag\(^{+}\)-C stability can help design more stable and reproducible nanostructures. For example, terminal C-Ag\(^{+}\)-C mismatches were used for creating Ag\(^{+}\) sensors [11,12].

The stability of terminal C-Ag\(^{+}\)-C mismatches has not yet been sufficiently studied. In this work, we investigated the ability of poly(C-Ag\(^{+}\)-C) formation between sticky polycytosine ends of short DNA duplexes. Figure 1 shows the model of the duplexes’ conjunction process. Duplexes with a different length of the cytosine ends were used to determine a sequence sufficient for binding and a duplex with no cytosine ends was used as a negative control. When linked, the duplexes form long structures with
regularly arranged silver ions. We believe that such objects can become an additional tool in the nanotechnology.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Scheme of the assembling of the oligonucleotides with the sticky poly-cytosine ends into longer structures due to the formation of bonds through silver ions.

2. Materials and methods

In the present work the following oligonucleotides were used:

- **1a:** 5'-TTTTTTTTTTATATATATATATATATATAT-3'
- **1b:** 5'-AAAAAAAAAAATATATATAAAAAAAA-3'
- **2a:** 5'-TTTTTTTTTTATATATATATATATATATATATATATATATAT-3'
- **2b:** 5'-AAAAAAAAAAATATATATAAAAAAAAACCC-3'
- **3a:** 5'-TTTTTTTTTTTATATATATATATATATATATATATATATATATATATATATATATAT-3'
- **3b:** 5'-AAAAAAAAAAATATATATAAAAAAAAACCCUCCCC-3'
- **4a:** 5'-TTTTTTTTTTTTATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATAT-3'
- **4b:** 5'-AAAAAAAAAAATATATATAAAAAAAAACCUCCCCUCCCC-3'
- **5a:** 5'-TTTTTTTTTTATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATAT-3'
- **5b:** 5'-AAAAAAAAAAATATATATAAAAAAAAACCCUCCCCUCCCCUCCCC-3'

These oligonucleotides were synthesized by Alkor Bio company.

For the charge screening, a solution of NaNO$_3$ with an ionic strength of 150 mM was used. AgNO$_3$ was used as a source of silver ions. It was dissolved in 150 mM NaNO$_3$ in the required concentration, thus when the solution of silver was added to the solution of duplexes, the ionic strength of the solutions did not change.

The systems preparing procedure for static light scattering experiments is described below. Two solutions of single-stranded oligonucleotides with equal concentrations were mixed and heated to a temperature above the melting point (approximately 50°C), then were slowly cooled to room temperature. After that, to precipitate impurities the solution of duplexes was purified in an ultracentrifuge for 10 minutes with an acceleration of 10,000 g and transferred to a clean DLS cuvette. AgNO$_3$ solution was also purified the same way. The final concentration of duplexes in solution was 4 μM.

The measurements were carried out using the Photocor setup at 20°C, the scattering angle was set to 90°, the wavelength of the laser was 657 nm.

Samples for atomic force microscopy were prepared similarly but without preliminary centrifugation. 2-5 μL of the solution was placed on the mica, then about of 10 μl of distilled water was added to the droplet, and after 2 minutes the remaining solution was blown off the surface with a blower. The experiments were carried out on the Bruker Multimode IV microscope.

3. Results and discussion

Firstly, the oligonucleotide polymerization was studied using static light scattering technique. The average scattering intensity was measured depending on the added amount of silver ions as shown in figure 2.
It can be seen that for solutions of duplexes with 6, 8 and 10 cytosines on the ends the significant increase of the average scattering intensity was found when the number of silver ions exceeded one ion per duplex. The increase in the scattering intensity indicates that larger objects are formed in the solution. That is presumably associated with the linkage of duplexes by the C-Ag\(^{2+}\)-C bonds.

![Figure 2. The dependence of the average scattering intensity on Ag\(^{+}\) concentration.](image)

Since it is known that under certain conditions silver ions can be reduced to form nanoparticles, it was additionally verified that the increase in the scattering intensity was not associated with the scattering by silver nanoparticles. For this, the UV spectrum of the solution was measured after the SLS experiment. The plasmon resonance peak on the wavelength of approximately 400 nm was not found on the spectrum, which means that there were no silver nanoparticles formed in the solution. This once again confirms that the increase in the scattering intensity is associated precisely with the formation of large structures when duplexes are linked through silver ions.

For duplexes without cytosine ends, no change in the scattering intensity was observed when silver ions were added. Thus, for this kind of duplexes, no large structures were formed with the addition of silver ions. That confirms the assumption that the formation of large structures is associated precisely with the cross-linking of cytosine sequences through silver ions with the formation of C-Ag\(^{2+}\)-C pairs, and is not associated with nonspecific aggregation of duplexes.

For duplexes with cytosine end sequences consisted of 3 cytosine bases, when 1 or 3.3 Ag\(^{+}\) per cytosine base were added to the solution, no significant changes in scattering intensity were observed. That shows that, probably, 3 cytosines is not enough to link two duplexes by C-Ag\(^{2+}\)-C bonds at 20°C.

Thus, it was shown that for duplexes with cytosine ends consisted of 6 bases or more, binding through C-Ag\(^{2+}\)-C pairs occurs with the formation of larger structures. However, the minimum length of poly-cytosine sticky ends required for joining duplexes was not determined yet.

Data obtained using atomic force microscopy, in general, are in agreement with the static light scattering results. The AFM images, obtained for a solution of duplexes with 6 cytosines without the addition of silver ions and with the addition of 6 silver ions per duplex are shown in figure 3.

It can be seen that the AFM image of the sample without silver ions contains only separately located small structures, that are probably single duplexes. After the addition of silver ions, the AFM image changed significantly – larger structures can be seen, which probably consist of linked duplexes. However, these structures do not look like long linear molecules, like natural DNA molecules, but more compact, "folded" structures. The crowding of the aggregates can be caused by the presence of many single-strand breaks, as follows from the polymerization scheme shown in figure 1.

It can be seen that with the addition of silver ions, the peak of the area distribution shifts to the right, particles with large area appear. The height distribution also shows an insignificant increase in structure heights. AFM images of duplexes with 10 cytosines look similar to the ones with 6 cytosines.
Figure 3. AFM images obtained for a solution of duplexes with 6 cytosines without the addition of silver ions (a) and with the addition of 6 silver ions per duplex (b). Corresponding area (c) and height (d) distributions of formed structures.

AFM images and distributions for duplexes without cytosines at the ends, however, did not change after the addition of silver ions. The AFM images of duplexes with and without silver ions look practically identically – only small single structures can be found, while the distributions practically coincide. That suggests that no larger structures are formed when silver ions are added to these duplexes. That confirms the assumption that in the absence of cytosine ends, duplexes cannot be joined into large structures, which means that assembly occurs precisely due to C-Ag⁺-C formation.

In general, the obtained results are consistent with the results reported previously [9], which described the formation of long poly-cytosine duplexes through multiple C-Ag⁺-C bonds in presence of silver ions. Although the studied systems were different, in our case the linkage of long cytosine sequences through the formation of C-Ag⁺-C pairs in solution was also shown. The process of linkage of cytosine sequences, in turn, leads to the conjugation of duplexes into longer DNA structures, which was studied in this work.

4. Conclusions
In this work, we showed the formation of large structures from the duplexes with sticky polycytosine ends upon the addition of silver ions due to the formation of C-Ag⁺-C bonds. That was confirmed for duplexes with ends consisted of 6, 8, and 10 cytosines via static light scattering and atomic force microscopy experiments.
For further research, it is of interest to study duplexes with sticky ends of 4 and 5 cytosines to determine the minimum required length through which the duplexes can be linked.

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