Revealing a possible sensor mechanism of DNA - based silver nanoclusters

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Abstract. The mechanisms underlying the sensory activity of silver clusters (Ag NCs) synthesized on oligonucleotide matrices are not understood yet. It is known that close proximity of cytosine and guanine rich sequences causes colour changes in the luminescence of Ag NCs. We studied two types of fluorescence activation of Ag NCs due to close proximity of two DNA strands. We concluded that the activation phenomenon could be explained by the same mechanism, namely by the reassembling of the silver atoms on the double-stranded DNA formed upon hybridization. Our results could be useful for further Ag NCs-based sensors development.

1. Introduction

Molecular detection in vitro and in vivo is one of the challenges of modern science. Luminescence based sensors are widely used in various biochemical and biomedical applications. Luminescent metal nanoclusters are a relatively new type of luminophores extensively studied last decade [1]. They have small size, high quantum yield and photostability. Among them, DNA-based fluorescent silver clusters (Ag NCs) are rapidly gaining attention in the fields of biosensors and biological assays for their applications in molecular detection [2-5]. In particular, DNA-stabilized Ag NCs can be used in DNA/RNA and gene detection [6]. The method is based on the ability to switch their fluorescence on and off or tune their color through interactions with a target DNA sequence. The origin of the most effects lies in a so-called guanine-proximity-induced fluorescence activation phenomenon [7-8]. Typically, dark non-luminescent Ag NCs are first synthesized on a NC-bearing cytosine-rich strand. Then, upon hybridization with a complementary enhancer strand containing a guanine-rich tail, a strong red fluorescence emission appears. In spite of the fact that this phenomenon was discovered ten years ago, the mechanism remains unknown. This hinders the application development. In a recent study [9], Petty et al. developed a DNA complex in which hybridization with a target DNA brought two single-stranded DNA fragments together. This allowed the synthesis of an emissive Ag cluster in the system. The authors suggested that it could serve as a basis for biosensors.

In this work, we investigated those systems in more detail. The aim was to shed light at the NCs fluorescence activation mechanisms. We synthesized Ag NCs on both the cytosine-rich and “guanine enhancer” strands and bring them together. The same experiments were performed with the other ssDNA fragments. Based on the results obtained, we concluded that the activation phenomenon can be explained by the same mechanism, namely by the reassembling of the silver atoms on the double-stranded DNA formed upon hybridization.
2. Experiment

The DNA sequences (purchased from Beagle, Evrogen, Russia) as well as the enhancer-proximity-induced fluorescence activation experiments are shown in table 1 and figure 1 respectively. DNA strands were mixed with Ag⁺ in sodium phosphate buffer (pH 6.6). Then freshly prepared NaBH₄ was added to DNA/Ag⁺ solution. Final concentrations were 15uM G-rich/C-rich DNA, 90uM AgNO₃, 90uM NaBH₄, 20mM sodium phosphate buffer for the G-enhancement experiments and 2.5uM DNA, 15uM AgNO₃, 7.5uM NaBH₄, 50mM sodium phosphate buffer for DNAx.1/DNAx.2 experiments. Luminescence spectra were recorded using a spectrofluorophotometer RF-6000 (Shimadzu). Absorbance spectra were acquired on a spectrophotometer Specord 210 Plus (Analytik Jena).

Table 1. DNA sequences used in this work.

| Names     | Sequences (from 5’ to 3’)                      |
|-----------|------------------------------------------------|
| C-rich DNA| CCCTTAATCCCTAATAATAATTTTAAATATTATTTAAT        |
| G-rich DNA| ATTAATAAATAATTTAATTATATTAGGGTGGGGTGGGGTGGGG   |
| DNAx.1    | CCCCACCACCAATACCCACCATCACA                     |
| DNAx.2    | AGGAGTCCTTCTGACAATCCCCTTTTT                   |
| Target DNA| TGTGATGGGTCAGAGAGGACTCCT                      |

Figure 1. Schematic showing the guanine-proximity (a) and two-stranded DNA (b) induced fluorescence activation experiments.

3. Results and discussion

3.1. “Guanine-proximity”-induced fluorescence activation.

In the first experiment, Ag NCs were synthesized on the C-rich sequence. After the G-rich sequence addition, a strong red fluorescence emission at 630 nm and a weaker emission at 560 nm appeared (figure 2, top). The red emission reached a maximum intensity in 1 h time and then started to decrease, while the green one increased. The corresponding evolution of the absorption spectra is presented in figure 3 (at left). As can be seen, upon addition of the G-rich DNA, the absorption of a dark Ag cluster at 420 nm decreases, while two new peaks of the green and red clusters appear at 490 and 560 nm respectively. In the next experiment, Ag NCs were first synthesized on the G-rich sequence, and then the C-rich DNA was added. Like in the previous case, the same red and green emissions are also observed (figure 2, bottom), although with lesser intensity. The corresponding absorption spectra are shown in figure 3 (a). In this case, another precursor, a dark cluster with the maximum at 380 nm, is destroyed, while the absorption shoulder at 490 nm is increased. The green cluster in these processes is a dominated product. This is also illustrated by the absorption spectra acquired in the case when the synthesis was performed after hybridization on the C-rich and G-rich strands (figure 3). Thus, the final products appear to be the same in both cases. The corresponding processes can be described by the following equations:

\[
\begin{align*}
C\text{-rich DNA-Ag} & \rightarrow C\text{-rich DNA-Ag-G-rich DNA} \\
C\text{-rich DNA-Ag-G-rich DNA} & \rightarrow C\text{-rich DNA-Ag-G-rich DNA}
\end{align*}
\]
\[ G\text{-rich DNA-Ag (380, dark)} + C\text{-rich DNA} \rightarrow C\text{-rich DNA-Ag-G-rich DNA (560 / 620)} + 
C\text{-rich DNA-Ag-G-rich DNA (490 / 560)} \] (2)

**Figure 2.** 2D fluorescence contour plot of C-rich DNA templated Ag NCs before (top left) and after (top right) G-rich sequence was added; G-rich DNA templated AgNCs before (bottom left) and after (bottom right) adding C-rich sequence.

**Figure 3.** Absorption spectra of C-rich (a) and G-rich (b) DNA-templated silver NCs before and after adding the second strand.

### 3.2. Two DNA strands proximity-induced fluorescence activation.

Similar experiments were performed with two other DNA sequences, designated as DNAx.1 and DNAx.2 in figure 1. In this case, the close proximity of the strands was achieved by the hybridization of both strands with a target DNA sequence (figure 1 (b)). First, an Ag NC was synthesized on the DNAx.1 and then the DNAx.2 was added. Next, vice versa, the synthesis of a dark cluster was performed on the DNAx.2 and then DNAx.1 was added. And finally, the clusters were synthesized after
hybridization of two strands. The corresponding fluorescence 2D contour plots and absorption spectra are presented in figures 4 and 5.

Figure 4. 2D fluorescence contour plot of DNAx.1 (top), DNAx.2 (middle), DNAx.1 and DNAx.2 mixture (bottom) templated AgNCs before (left) and after (right) adding the other DNA strand.

In all cases, a dominant green-emitting cluster was formed. The first two cases open a new fluorescence light-up sensor for the detection of a target DNA. The main processes can be described by the following equations:

\[
\text{DNAx.1-Ag (400, dark) + DNAx.2 → DNAx.1-Ag-DNAx.2 (490 / 560)}
\]  
\[
\text{DNAx.2-Ag (420, dark) + DNAx.1 → DNAx.1-Ag-DNAx.2 (490 / 560)}
\]
These experiments lead to two main conclusions. First, the fluorescent Ag clusters can be formed efficiently only when two DNA strands are in close proximity. Second, the same fluorescent Ag clusters are formed from different dark Ag precursors (dark clusters) located on different DNA strands. The final result depends on the sequences of both strands stabilizing the fluorescent cluster. Thus, the “emission activation” is not a simple step providing of a G-rich DNA or another DNA proximity, but is related to a reassembling of Ag atoms on a new two-stranded DNA template and to the formation of the new clusters.

4. Conclusions
We have studied two cases of fluorescence activation of Ag NCs. We conclude that the phenomena of both the so-called G-enhancement and the light-up of a green-emitting Ag cluster induced by the proximity of two DNA strands can be explained by the same mechanism. This activation mechanism includes a reassembling of Ag atoms on the new two-stranded DNA templates and thus formation of the new fluorescent clusters. We have also designed a new light-up sensor for the detection of a specific DNA sequence inspired by the findings of work [9].

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References
[1] Chakraborty I and Pradeep T 2017 Chem. Rev. 117 8208-71
[2] Yuan Z, Chen Y-C, Li H-W and Chang H-T 2014 Chem. Commun. 50 9800-15
[3] Gwinn E, Schultz D, Copp S and Swasey S 2015 Nanomaterials 5 180–207
[4] Ramazanov R R, Sych T S, Reveguk Z V, Maksimov D A, Vdovichev A A and Kononov A I 2016 J. Phys. Chem. Lett. 7 3560–6
[5] Chen Y et al. 2018 Acc. Chem. Res. 51 11 2756-63
[6] Obiososca J M, Liu C and Yeh H-C 2013 Nanoscale 5 8443–61
[7] Yeh H-C, Sharma J, Han J J, Martinez J S and Werner J H 2010 Nano Lett. 10 8 3106-10
[8] Yeh H-C, Sharma J, Shih I M, Vu D M, Martinez J S and Werner J H 2012 J. Am. Chem. Soc. 134 11550-8

Figure 5. Absorption spectra of DNAx.1 (a), DNAx.2 (b) and their mixture (c) templated AgNCs before (blue) and after (red) adding the other DNA strands.
[9] He C, Goodwin P M, Yunus A I, Dickson R M and Petty J T 2019 *J. Phys. Chem. C* **123** 28 17588-97.