論 文 の 内 容 の 要 旨

論文題目　Fabrication of DNA Nanostructure and Its Applications
（ＤＮＡナノストラクチャの構築とその応用）

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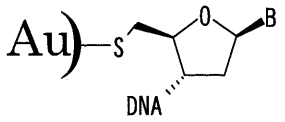
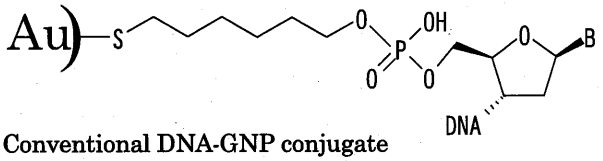
Introduction

DNA plays an important role in biochemistry and biology. Numerous applications of this chain molecule were found not only in life science but also in nano science. DNA-modified gold nano particles (GNP) attracted tremendous interests in recent years. Various genetic diagnostics technologies were developed and numerous nano structures were fabricated with them. To immobilize DNA to gold surface, various mercapto modified DNA were employed. All the previously employed DNA-SH own an alkyl linker between DNA backbone and mercapto group, which made the DNA-Au system more complicated. The hydrophobic alkyl linker can partly change the property of DNA, for example, electrical characteristics or molecular flexibility. Moreover, the alkyl linker used for this indirect attachment often diminishes the transfer of signals from DNA to gold interface (and vice versa) and thus their removal is desirable. In this thesis, DNA-GNP conjugates and Au-DNA-Au nanostructures are fabricated as shown in **Scheme 1**. By using 5'-mercapto-5'-deoxythymidine, DNA is directly attached to gold particle surface to obtain a new type DNA-Au conjugate. This new type DNA-GNP conjugate showed good hybridizing specificity in Au-DNA-Au nanostructure fabrication. The obtained Au-DNA-Au nanostructure showed higher ability in electron transfer, which indicates

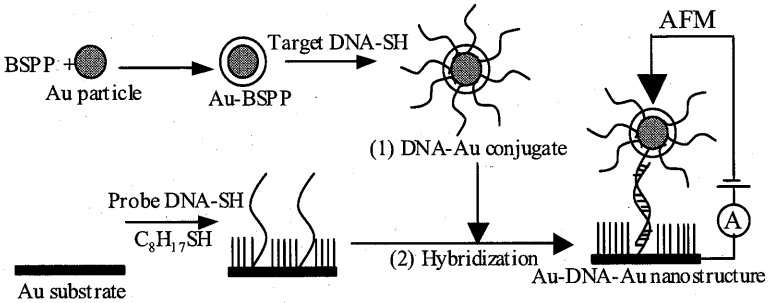


New type DNA-GNP conjugate



Conventional DNA-GNP conjugate

Fig. 1 Structures of two kinds of DNA-gold nano particle (GNP) conjugate



Scheme 1. The method developed to fabricate DNA-GNP conjugate and Au-DNA-Au nanostructure

promising applications for information transmission along DNA chains.

Experimental

Typical sequences of the oligonucleotides used in this study are as follows. DNA1: 5'-HS-TACCAGGATTACCGCTCACA, DNA2: 5'-HS-TGTGAGCGGTAATCCTGGTA, DNA3: 5'-HS-C6-TACCAGGATTACCGCTCACA (bearing a -C_6H_{12}- linker between its mercapto group and DNA backbone), DNA4: 5'-HS-C6-TGTGAGCGGTAATCCTGGTA DNA5: 5'-TACCAGGATTACCGCTCACA (a native DNA for control),

Gold particles (0.009 μM, d=10 nm) were coated by BSPP (bis(p-sulfonatophenyl) phenyl phosphane) in 400 μM BSPP solution for 1h. The Au-BSPP conjugates were separated by centrifuge. DNA-GNP conjugates were obtained by treating Au-BSPP conjugates (0.05 μM) with 0.5 μM DNA-SH at a specific pH for 24 h. In order to form a self assembled monolayer (SAM), the probe DNA-SH of 0.1 μM, was codeposited to gold substrates with octanethiol (10 μM) and the solution was kept for 1 h. Au-DNA-Au nanostructure was obtained by hybridization of the immobilized probe DNA with their complementary DNA-GNP conjugates (0.01 μM) for 3 h. Atomic force microscopy (AFM) was employed to observe the Au-DNA-Au nanostructure and measure their electric properties.

Results and Discussion

1. Fabrication of the new type DNA-GNP conjugate

The DNA-GNP conjugate is the key intermediate for various DNA-GNP applications. For the first time, DNA was directly attached to GNP surface without any alkyl linker. The formations of our new type DNA-GNP conjugates were studied by electrophoresis.

Attachment of DNA to GNPs

It was found that both the DNA-SH (DNA1 and DNA3) and native DNA (DNA5) attached to gold particles easily. Fig.2 shows the amount of free DNA in solution after incubation with GNPs by electrophoresis. After incubation of 1 h with GNPs, the amount of the three DNA in solution decreased (Lane 1, 2, 3) indicating all of the three types of DNA partly attached to gold particles. After 24 h incubation, most of the DNA attached to gold particles (Lane 4, 5, 6). The residual of the free DNA in the solution was almost undetectable. Such kind of nonspecific attachment should be avoided in

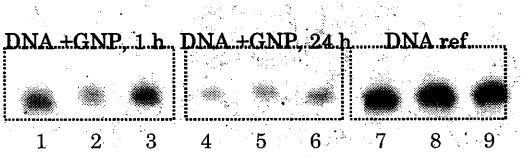


Fig.2 Nonspecific attachment of three DNA to GNPs

Lane1,4: 0.1 μM DNA3 + 0.01 μM GNPs; Lane2,5: 0.1 μM DNA1 + 0.01 μM GNPs; Lane3,6: 0.1 μM DNA5 + 0.01 μM GNPs; Lane7: 0.1 μM DNA3; Lane8: 0.1 μM DNA1; Lane9: 0.1 μM DNA5

DNA-GNP conjugate fabrication.

BSPP protected GNPs : Protect GNP from nonspecific attachment of DNA

It was reported that BSPP could attach to GNP by coordinated bonds. The BSPP protected GNPs (Au-BSPP conjugate) has many advantages. 1. The GNPs are stabilized against aggregation since many negative charges caused by BSPP. 2. The Au-BSPP has a good mobility in electrophoresis that attachment of DNA to it can be easily monitored. We suspect that the Au-BSPP can prevent the nonspecific attachment of DNA from GNPs. Only the DNA-SH can substitute the BSPP molecules by the more stable DNA-S-Au linkage. It is known that GNPs will show a lower mobility when attached by DNA. The disappearance of free DNA bands also provides good evidences for the DNA-GNP conjugates formation. As Fig. 3 shows, native DNA doesn't attach to BSPP protected GNP after 24 h incubation. So with BSPP protection nonspecific attachment of DNA to GNPs can be successfully prevented.

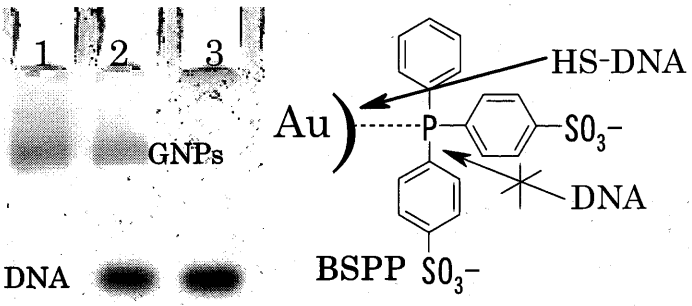


Fig.3 Prevent nonspecific attachment of DNA to GNPs by BSPP protection

Lane1: Au-BSPP; Lane2: 0.5 μM DNA5 + 0.05 μM Au-BSPP; Lane3: 0.5 μM DNA5

1.3 pH dependence in DNA-GNP fabrications

The Au-BSPP conjugates were employed for the fabrication of DNA-GNP conjugates. As Fig.4 shows, astonishingly, at pH 8, DNA1 or DNA5 doesn't attach to gold particles (lane 3 and 4), although the DNA3 (with a linker) attaches to the gold particles very well (lane 2). When the pH was 4.0 or lower, however, both the DNA1 and DNA3 are effectively bound to the gold particles (lane 5 and 6). Since DNA5 (without a mercapto group) doesn't attach to gold particles at any pH (lane 4 and 7 or even lower), the possibility of nonspecific attachment can be ruled out. The different behavior of the two types of DNA-SH (with or without an alkyl linker) is attributed to the electrostatic repulsion between the negatively charged Au-BSPP and DNA-SH. Because of the absence of a linker, DNA1 can't approach Au-BSPP to an accessible distance. Accordingly, an acidic pH is necessary to decrease the negative charges of both Au-BSPP and DNA1 to suppress the electrostatic repulsion.

The charges on DNA1 or Au-BSPP were directly monitored by electrophoresis at

various pH. As Fig.5 shows, Both DNA1 and Au-BSPP hold much less negative charges at acidic pH indicated by their mobility in electrophoresis. When pH is 2, the negative charges on DNA1 or GNP almost decreased to 0.

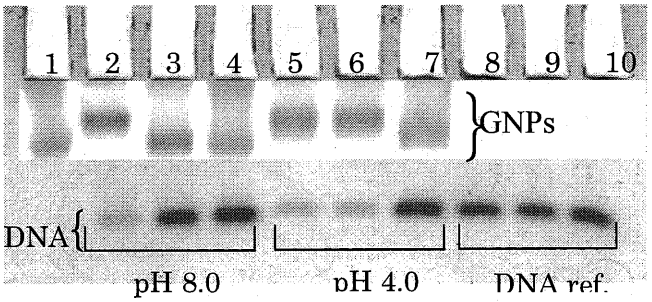


Fig.4 Electrophoresis of DNA-Au conjugate fabrication

Lane 1: Au-BSPP, lane 2, 5: DNA3 + Au-BSPP, lane 3, 6: DNA1 + Au-BSPP, lane 4, 7: DNA5 + Au-BSPP, lane 8: DNA3, lane 9: DNA1, lane 10: DNA5.

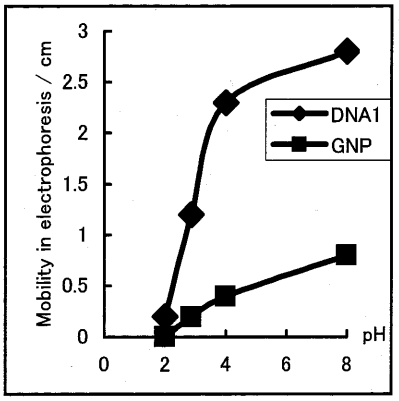


Fig.5 Mobility of DNA1 and GNP at various pH

Further study showed the base contents of DNA-SH would strongly influence its dependence to pH in DNA-GNP conjugate fabrication. **Table 1** lists the pH necessary for various DNA-SH to obtain DNA-GNP conjugate. As expected, polyC-SH and polyA-SH can attach to BSPP protected GNPs much more easily than polyG-SH and polyT-SH. The result agrees with our hypothesis about DNA-GNP conjugate formation since the bases with higher pKa can be more easily protonated.

Table. 1 The pH necessary to fabricate DNA-GNP conjugates and pKa of the four bases

DNA	pH necessary	pKa of bases
DNA1	< 4	
polyC-SH	< 7	C: 4.56
polyA-SH	< 4	A: 3.88
polyG-SH	< 2	G: 3.6
polyT-SH	< 2	T: no

2. Fabrication of Au-DNA-Au nanostructure

The most important property of DNA-Au conjugate is its hybridizing specificity. The obtained DNA-GNP conjugates were employed to hybridize with their complementary DNA probes, which were immobilized on Au substrates. AFM assay shows that many DNA-GNP conjugates specifically hybridized with its complementary probes on the Au substrates (**Fig. 6A**). The observed GNPs have a height of 8-10 nm, which agrees with the size of gold particles employed.

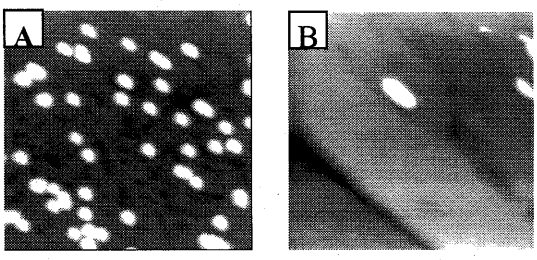


Fig. 6 AFM images of Au-DNA-Au nanostructure (500×500 nm)

(A) DNA-GNP conjugates (DNA1-Au) hybridized with its complementary DNA (DNA2).

(B) DNA-GNP conjugates (DNA1-Au) treated with

noncomplementary DNA (DNA1).

In contrast, almost no GNP was immobilized when the DNA probes on the gold substrate were not complementary with the DNA-GNP conjugates (**Fig. 6B**). The fabrication of the Au-DNA-Au nanostructure is reliable and reproducible.

The influence of the conditions for the Au-DNA-Au nanostructure fabrication was studied. It was found that the amount of immobilized GNPs is proportional to the concentrations of probe DNA in low concentrations (**Fig. 7**). This result indicates the immobilization of gold particles is concretely caused by the hybridization of the probe DNA and DNA-GNP conjugate. When the concentration of probe DNA-SH exceeded 0.1 □M, the amount of the immobilized gold particles kept constant. Some other parameter seems to be more determinative when there are enough probe DNA on gold surface.

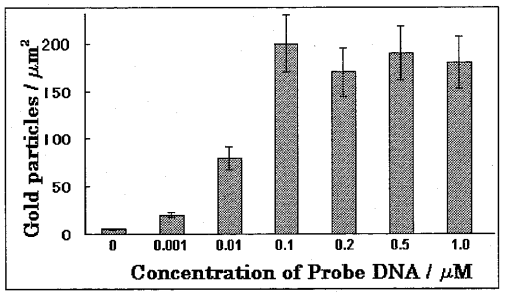


Fig. 7 Influence of Probe DNA concentration to Au-DNA-Au nanostructure fabrication

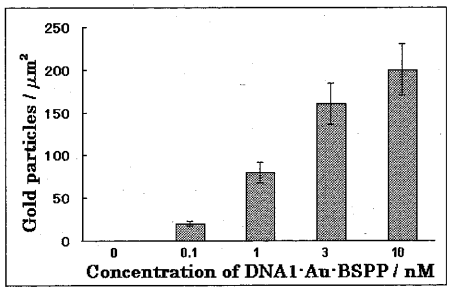


Fig. 8 Influence of DNA1-GNP conjugate concentration to Au-DNA-Au nanostructure fabrication

The influence of the DNA-GNP conjugate concentration to the Au-DNA-Au nanostructure is shown in **Fig.8**. More GNPs were immobilized as the concentration of DNA-GNP conjugates increased. The red color of the DNA-Au conjugate didn't change obviously after hybridization, suggesting that only very small part of the DNA-GNP conjugates hybridized with the probe DNA immobilized on gold substrate.

3. Electro characterization of Au-DNA-Au nanostructure

The obtained Au-DNA-Au nanostructure was subjected to conductive probe AFM (cp-AFM) measurement. As shown in **Scheme 1**, the $C_8H_{17}SH$ SAM is expected to serve as an insulator layer. When a voltage was applied between the GNP and the gold substrate, the current through the DNA chain was recorded. For each sample, at least 200 randomly selected points were measured. The U-I curve of these points varied very much. **Fig.9** shows the distribution of the currents under 100 mV.

The cp-AFM measurement of $C_8H_{17}SH$ SAM for its conductivity was also measured, resulting currents below 1 nA under 100 mV, which agrees with the $C_8H_{17}SH$ SAM conductivity measurements by literature (*JACS*, 123, 5549). In control experiment when the probe and target DNA are noncomplementary, the obtained DNA-$C_8H_{17}SH$

SAM also showed the same conductivity (I < 1 nA). This result indicates that the DNA-$C_8H_{17}SH$ SAM wasn't damaged by the DNA-GNP solution.

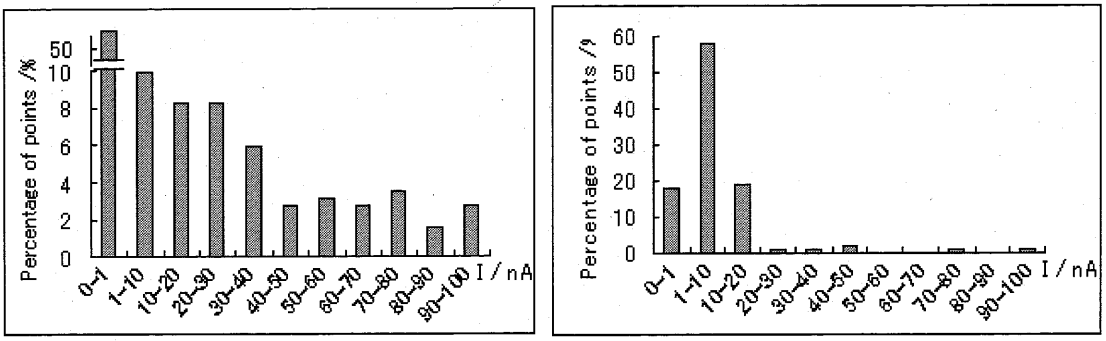


Fig.9 (A) Current of Au-DNA-Au nanostructure (without a linker) under 100 mV. **(B) Current of Au-DNA-Au nanostructure (with a linker) under 100 mV.**

As **Fig.9 (A)** shows, when Au-DNA-Au nanostructure was measured, about half of the points showed currents lower than 1 nA, which can be presumed as the positions that no Au-DNA-Au nanostructure was fabricated. Neglecting the currents below 1 nA, the conductivity of the 20 mer ds-DNA (without a linker) can be calculated to 10-1000 nS, while the corresponding DNA with a -C_6H_{12}- linker showed a conductivity of 10-200 nS. The DNA without a linker showed a comparably higher conductivity than that with a linker or $C_8H_{17}SH$.

Conclusions

DNA was directly attached to gold surface through S-Au bonds to fabricate a new type DNA-GNP conjugate and the corresponding Au-DNA-Au nanostructure. pH was found to play an important role in DNA-GNP conjugate fabrication. The different behaviors of different DNA-SHs in their DNA-GNP conjugate fabrication were contributed to their molecular charges. The obtained Au-DNA-Au nanostructure was observed by AFM. Furthermore the electric properties of DNA were studied by cp-AFM. Duplex DNA without a linker showed a higher conductivity than that with a linker, which suggests our new system is more promising as molecular wires for charge transmission along DNA chains.